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MICROBIOLOGICAL ACTIVITY, AND THE EFFECTS OF FERTILIZER ON  
REACTION OF WHEAT TO OPHIOBOLUS GRAMINIS, IN UNSTERILIZED  
AND RECONTAMINATED STERILIZED SOILS

Gwynfryn Richards

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INTRODUCTION

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The plant pathologists have observed and obtained evidence that some soil fungi and bacteria are capable of suppressing the virulence or growth of pathogenic organisms in the soil. Therefore, to understand soil borne plant diseases, it becomes necessary to understand the pathogen's life in the soil, including the relationships to other soil micro-flora and micro-fauna. Since the soil is a very complex environment it would be difficult to study the pathogen along with its associative and antagonistic organisms in the normal soils. In order to use soil for the study of micro-organisms, it becomes desirable to remove its natural micro-population, which is done by one of the several methods employed for sterilization. When soils are sterilized it has been found that their chemical and physical nature is changed. These changes must be understood, as an alteration in environment markedly affects any subsequent microbiological growth according to Waksman (31) and others.

Malowany (14), using Alberta soils, found that when they were steam sterilized and recontaminated the biochemical activity of the soil population was much more pronounced, but



counts of the bacteria, fungi and protozoa were not carried out and for this reason microbiological counts were made in this investigation. Malowany (14) also did sufficient work on the physical and chemical changes to allow the writer to commence a study of antagonistic and non-antagonistic organisms in relation to the ~~"take-all" disease of wheat~~, Ophiobolus graminis.



### Review of Literature

It is not intended to give a complete review of the literature on the effects of steam sterilization of soil or on the associative and antagonistic relationships of micro-organisms in the soil, but merely to review papers closely related to the work that is under consideration. There is not only extensive literature on the inter-relationships of the soil population and the soil borne pathogens but also several good reviews by Waksman (28), Porter and Carter (18) and Garrard and Lochhead (8). The effects of sterilization of soils have been ably covered by Malowany (14).

With studies conducted on artificial media it has often been found that an inhibitory effect is exerted by one organism on another. Henry (10) was one of the first to investigate the inhibitive action of micro-organisms on plant pathogens in the soil. He observed that the wheat foot rotting pathogen, Helminthosporium sativum, was suppressed by the natural micro-flora of a black soil from the Edmonton district. Sanford and Broadfoot (20) found that inoculum of Ophiobolus graminis decreased in efficiency regardless of the amount used when introduced into the soil. They came to the same conclusion as Henry (10), but carried the work further by isolating on artificial media, fungi, actinomycetes and bacteria. They observed that many of these cultures or the culture filtrates suppressed the pathogenicity of Ophiobolus graminis while some increased its virulence. Henry (11) has shown that infection of wheat seedlings with



Ophiobolus graminis varies greatly, depending upon whether the soil was sterilized or not. The non-sterilized soil gave protection at temperatures above 20°C. but not below, while the sterilized soil gave little protection at any temperature. Broadfoot (1) with a similar experiment, also showed that the infection rating was higher in the sterilized than in the non-sterilized soil.

Garrett (7) found that Ophiobolus graminis grows along the roots of wheat plants best of all in sand, and that its growth in soil is favored by any condition tending to promote better aeration and by steaming, except in the case of the more acid soils. With the better aerated soils he believed that the fungus had a better growth due to the removal of carbon dioxide. To substantiate these findings he used an acid soil which he aerated and was able to produce a greater infection than on the check.

Fellows (5) was of the same opinion as Garrett. He, therefore, grew Ophiobolus on solid and liquid media, in atmospheres with varying concentration of oxygen and carbon dioxide. The fungus grew equally well at most concentrations of carbon dioxide except the upper limits (18%), and that a very small <sup>change in</sup> percentage of oxygen greatly reduced growth. He ~~therefore~~ concluded that variations in carbon dioxide and oxygen as found in arable soil are not great enough to affect materially the growth of Ophiobolus graminis. Machacek (13) observed that unusually severe infection by Helminthosporium and Fusarium occurred in fields of wheat where the concentrations of soluble salts were



high.

Fellows (4 ) in studying certain soil phases of the wheat "take-all" problem, found that the application of horse and chicken manure, alfalfa stems, boiled barley and oat mixtures, both under greenhouse and field conditions, reduced the severity of the disease. The results may be explained in many ways; the following may have occurred: the manure increased the numbers and activities of common competitive organisms which inhibit the growth of root-rot fungi; or, the increased activity resulted in a decrease of available nitrates. Vanterpool (26) working with Pythium root-rot, found that a high nitrate combined with a low available phosphate generally resulted in greater disease severity, and that fields with a narrow nitrate to available phosphate ratio were less severely attacked.

Since ammonia is a decomposition product of manure, Neal et al (17) attempted to find its effect on the cotton root-rot fungi. Using a nutrient solution containing various sources of nitrogen, he found that with ammonium nitrate and ammonium sulphate there was little growth of mycelium. With the nitrates of calcium, sodium and potassium abundant growth was produced. The concentration of nitrogen in all sources was 12.4 grams per litre.

McLean and Wilson (15) and others found the soil fungi to be very strong ammonifiers, most of them liberating greater amounts than the strong ammonifying bacteria. There were also differences in ammonifying power of different species of fungi. Coleman (2) found that the activities of soil fungi are governed by the type of soil and the quality of the organic matter, and



that with pure cultures each fungus will do best with a definite combination of soil and organic matter. Some of the organisms he tested were greatly benefitted by an increase of oxygen, others very slightly, while some gave no response, as measured by the ammonia producing ability. Optimum moisture requirements varied with the cultures of fungi under consideration. The studies of associated activities of known cultures of bacteria and fungi indicated that their combined activities were greatly accentuated or depressed and that an organism's activity in pure cultures was no indication of its behavior when introduced into other cultures. Waksman (29) found that the ammonifying power of fungi varied with the length of time they were grown on artificial media. It was found with organisms that were isolated for six months that their ammonifying power was increased, while ~~that~~ <sup>of</sup> others ~~were~~ <sup>was</sup> decidedly decreased.

Gainey (6) found a marked similarity for the curves of carbon dioxide and ammonia from soils, even when the data secured ~~were~~ obtained under anything but similar conditions. He did find that insufficient moisture affected both the gases, especially the  $\text{NH}_3$ , and that the optimum moisture for production of  $\text{NH}_3$  was considerably higher than that for carbon dioxide, but the carbon dioxide also dropped considerably if the moisture was below the optimum. There was a greater production of both carbon dioxide and ammonia if the system was continuously aerated, but the volume of air had no effect. Insufficient aeration caused a depression or delay in production of both gases. Semenuik (21) found there was no definite relationship between the organic



matter content of three Alberta soils (black, brown, gray), the nitrogen content, and the carbon dioxide evolved. Wollny (1880) (33) in experiments with horse manure and sand, observed that mixtures containing the larger amounts of the manure gave increased carbon dioxide up to a certain limit, beyond which little effect was found. This was attributed to the antiseptic properties of carbon dioxide. Horse manure treated separately with mercuric chloride, thymal, phenol, and heated for six hours at  $115^{\circ}$  gave off comparatively little carbon dioxide, showing that most of the carbon dioxide arose from the activities of micro-organisms. He also noted that increases in temperature and moisture up to certain limits increased carbon dioxide production. Stoklasa and collaborators (24,25) did further work on the problem of carbon dioxide production in soils by laboratory experiments. A sterilized soil was found to produce no carbon dioxide, and the evolution of carbon dioxide was inhibited by anaerobic conditions, and increased by fertilization with stable manure, by raising the temperature up to  $35^{\circ}$ , and by increasing the moisture in the soil up to 50% of its water holding capacity. They concluded that the amount of carbon dioxide evolved was dependent on the mechanical condition of the soil and its fertility. Intensity of carbon dioxide production showed the presence, not only of active bacteria, but also of easily available organic matter.

Van Suchtelen (27) treated soil with carbon disulphide and this resulted at first in greatly lowering the carbon dioxide production, which later increased until at the end of sixteen days the ratio of treated to untreated soil was 7.2 to 6.0.



From further work he concluded that the comparison of the carbon dioxide production of different soils furnishes a better means for the estimation of their relative bacterial activity than their bacterial counts.

Partial sterilization of soils by both heat and chemicals has resulted in tremendous increases in microbial numbers (19,32). Ludwig (12) working with sterilized recontaminated soils found that this treatment had the same effect as partial sterilization.



### Outline of Investigation

The investigation was divided into two phases: first, the microbiological changes, and secondly the chemical-biological changes effected by steam sterilization and recontamination in three typical Alberta soils. Co-operative experiments were also carried out in conjunction with the Department of Field Crops, Division of Plant Pathology, on the reaction of wheat to Ophiobolus graminis in pot cultures of unsterilized and recontaminated steam sterilized soils plus organic and inorganic fertilizers in Edmonton black loam and Fallis gray wooded silt loam.

The microbiological studies were confined to plate counts of bacteria, fungi, and protozoa in non-sterilized as compared with steam sterilized recontaminated soils.

The chemical-biological phase included the study of ammonification and carbon dioxide production by pure cultures in soil, carbon dioxide production from steam sterilized recontaminated soils as compared with the unsterilized, the effects of organic and inorganic fertilizers on nitrification and development of wheat seedlings in unsterilized and steam sterilized recontaminated soils uninfested and infested with Ophiobolus graminis, and the immediate effects of steam sterilization on ammonification.

The study of the micro-flora was commenced in November, 1938. The three soils were studied for a period of 22 weeks. The set-up consisted of 72 tumblers, with perforated covers, containing 100 grams of soil (water-free basis). This resulted in 12 tumblers for each treatment and each soil. That is, one-half of the tumblers were sterilized and recontaminated with the normal



flora. They were incubated in a dark chamber, cultivated and brought up to optimum moisture every ten to fourteen days. Plating was carried out immediately after sterilization and each week for six weeks. They were then plated twice at two week intervals and four times at four week intervals.

The protozoa counts were not satisfactory from this set-up, consequently a similar experiment was commenced in October 1939, in an attempt to get more satisfactory results.

Three ammonification experiments with pure cultures were conducted. The first was started in October 1938, and the second in March 1939; in both experiments 50 grams of Edmonton black loam (water-free basis) were used, and conducted for a period of 22 weeks. The soil was cultured in 96 (250 cc.) flasks, 24 being used for each pure culture of fungus used, thus allowing the use of two flasks for each determination. The flasks were plugged with cotton batting, into which glass rods had been rolled to allow cultivation of the soil.

The third ammonification/<sup>experiment</sup> was commenced in November 1939 with Fallis gray wooded soil, 100 grams (water-free basis) being used. The same number of flasks was used. It was found impossible to maintain the flasks at optimum moisture by using the type of plug used in the two previous experiments. This was probably due to the Fallis gray wooded soil containing less organic matter than the Edmonton black loam. The type of plug was therefore changed to a number five rubber stopper wrapped in cotton batting, which allowed for a transfer of air but to a more limited extent, and resulted in less moisture being lost. Organism number 32 was used in each of the three experiments to allow for



comparison of results. The flasks were maintained at optimum moisture, with the necessary precautions being taken to prevent them becoming contaminated.

Four complete experiments were conducted on the carbon dioxide production in black soils. Two were carried out with pure cultures of fungi, the second of which was a duplicate, but carried out at a later date. The remaining two were carried out on the three soils, comparing the unsterilized with the steam sterilized recontaminated. As in the first instances, these experiments were done in duplicate and at different dates.

The plan followed was the same in the four experiments. In the first, which was commenced on January 20th 1939, fourteen 500 cc. suction flasks, each containing 200 grams of Edmonton black soil (water-free basis) were sterilized, and flasks contaminated with the following cultures in duplicate: Ophiobolus No. 4, fungi Nos. 3, 19, 32, 44 and 1003. The remaining two flasks were not contaminated but were used as checks. They were all incubated for two weeks, when one of each culture was removed and the carbon dioxide production determined by aerating for forty-five minutes every twenty-four hours for a period of fourteen days. They were then discarded and the remaining seven flasks were used to carry on the study for the next twenty days.

The second, or duplicate experiment, was commenced on March 11th, 1939, and allowed to incubate eleven days, when half the flasks were removed and their carbon dioxide production measured for seventeen days. They were then replaced with the remaining seven flasks for a period of sixteen days.



The third and fourth experiments had a total of fourteen flasks each. Four flasks were used for each soil, two of which were sterilized and recontaminated, the other two being left to represent normal soil. The remaining two flasks contained Edmonton black soil which was infested with fungus No. 32.

The third experiment was started on October 26th, 1939, and was incubated for ten days before determining carbon dioxide production. Seven flasks, one of each treatment, were removed and the carbon dioxide production measured for a period of twenty-three days. They were then replaced with the remaining seven flasks which were used for twenty days.

The fourth experiment was set up on January 5th, 1940. The incubation and carbon dioxide determinations were carried out for the same periods as the set-up of October 26th, 1939.

Organism No. 32 was used in each of the set-ups to allow for comparison of data, and also as a means of indicating when contamination had taken place. No further moisture was added after bringing the soils to the optimum moisture at the beginning of the experiments, other than that brought in by the moist air during the aeration of the set-up while determining the carbon dioxide.

The study of the immediate effects of steam sterilization on ammonia production included the three soils. A total of six flasks was used, two for each soil, one for each treatment, each containing 25 grams of soil (water-free basis). They were brought to optimum moisture, allowed to stand twenty-four hours and sterilized for one hour, and the ammonia content determined immediately.

Greenhouse experiments were carried out to determine



the effects of several organic and inorganic fertilizers on the reaction of wheat to Ophiobolus graminis in pot cultures of unsterilized and recontaminated steam sterilized soils. In the first carried out, Edmonton black loam was used, starting in January 1939, and Fallis gray soil for the second, commenced March 1939, this being the essential difference between the two experiments. Three different forms of organic matter were used, namely finely ground alfalfa, wheat straw and poplar sawdust, separately, and in combination with ammonium phosphate (16-20), which also was applied alone. The amendments plus a small amount of unsterilized soil were mixed thoroughly with the soil in the top half of each pot. All pots were then incubated at approximately 20°C. for a period of three weeks. Inoculated series were replicated eight times in the first experiment and seven times in the second, with the exception of the series containing wheat straw, which had eight replicates. At the end of three weeks the pots were all sown to wheat and 40 grams of Ophiobolus graminis inoculum added, directly below the seed, to the series that were to be inoculated. (Four weeks were allowed to elapse between the setting up and the time of seeding and inoculating in the second experiment). After four weeks the crop was harvested and notes were taken on the plants for disease rating, height and total dry weight.

In October 1939 a third greenhouse experiment was commenced using Fallis gray soil. The amendments used were ammonium phosphate (16-20), straw rotted and unrotted, alone and in combination with ammonium phosphate. The pots were all prepared



at the same time, the soil necessary to fill half of the pots, which were to be sterilized, was sterilized in bulk and then transferred to the pots. Fresh wheat straw finely ground was then added to the soil in the pots that were to rot before seeding. Three weeks later the pots were all seeded and straw added to the series that were to contain unrotted straw. Ammonium phosphate was added at seeding time to the series that were to contain this treatment. The infested series were replicated eight times, the infestation being done as in the previous two experiments; the uninfested were replicated four times. The wheat seedlings were harvested four weeks later, the same data being taken as in the previous experiments. The pots were sown again to wheat, harvested four weeks later and records taken.

In the summer of 1939 a field experiment was conducted on the Fallis gray soil to study the effects of certain fertilizers which had previously reduced the percentage of disease and increased the yields of wheat seedlings in pot culture experiments.

Randomized rod row plots were seeded to Reward wheat. Chopped straw was added to the straw plot rows on April 25th and covered with soil. The wheat was sown May 1st and certain plots were artificially infested with Ophiobolus graminis at this time. Ammonium phosphate, where applied, was drilled in with the seed. The crop was harvested August 11th, and the average yields (per row) and percentage disease noted.



### Method of Analysis

The soils used in this investigation were collected from the three major soil color zones in the province of Alberta. They were collected during the summer of 1937 and 1938 in sufficient quantity to last the duration of the experiment and represent the surface 6 2/3 inches. The locations, color and textures are as follows:

Edmonton	Black	Silt loam to loam
Gros Ventre	Brown	Loam
Fallis	Gray Wooded	Silt loam.

All soils were air dried and sieved to remove extraneous materials.

Since this investigation is a continuation of that done by Malowany (14), the same optimum moisture and method of sterilizing the soil was followed. One slight modification was used in all cases where soils were to be sterilized--they were brought to optimum moisture twenty-four hours previous to steam sterilizing.

When sterilizing the soil for the co-operative experiments with the Department of Field Crops, University, on the effect of Organic and Inorganic Fertilizers on Reaction of Wheat to Ophiobolus graminis, the pots were thoroughly watered, prior to sterilizing for eight hours at fifteen pounds pressure.

The numbers of bacteria and fungi as determined by the plate colony count method were determined in normal soil and steam sterilized recontaminated with normal flora. Waksman and Fred's (30) sodium caseinate agar medium was used for bacterial counts and Czapek's sodium nitrate-sucrose agar medium for fungal counts. Bacterial counts were made on the seventh day and fungi



counts on the third day after plating. Plating for bacteria was done with a dilution of 1:100,000, <sup>for</sup> fungi <sup>with a</sup> ~~platings from~~ 1:10,000 ~~dilution~~ for the normal soil and <sup>a</sup> 1:100,000 for the sterilized recontaminated. The plates were poured in four replicates and the average used in reporting the results.

The chief disadvantages of the culture plate method are:

1. There is no single plating medium that will enable all the different soil bacteria to grow;
2. Each colony may represent clumps instead of single cells or spores, and in the case of fungi it is not known whether the colonies found have developed from broken pieces of mycelium or dormant spores or mixtures;
3. The counts obtained from different dilutions show no mathematical correlation.

In spite of these weaknesses this is the most practical method, as we are not concerned with total counts but with the relative numbers of <sup>micro-organisms in</sup> the unsterilized soil <sup>and</sup> ~~populations in relation~~ to steam sterilized and recontaminated soils.

Protozoa counts were also made as well as bacteria and fungi. Plating was done on a soil extract agar as recommended by Dixon (3). Since there were three different soils used, it necessitated the preparation of three media. This soil extract media was used, as Dixon found that it gave a more representative record of all classes of protozoa, but particularly of the amoebae and ciliates. The numbers of flagellates were sometimes less than the numbers found when other media were used. The plating was done from dilutions of 1:1,000, 1:10,000 and 1:100,000; three plates



being inoculated for each dilution. A 0.75% salt (NaCl) solution was used to moisten the plates and sterilized distilled water was added to maintain the moisture. Flat slide mounts were made at the end of four weeks, observations made and recorded.

A 0.75% salt (NaCl) solution was used for all the dilutions.

Ammonia was determined by the McLean and Robinson (16) method, twenty-five grams of soil (water-free basis) were leached with 500 cc. of 15% sodium chloride solution and the resulting extract distilled with magnesium oxide, collecting the ammonia in standard acid and titrating the excess with a standard base, using methyl red. Distillation was carried on until approximately 250 cc. distillate were collected.

Nitrates were determined colorimetrically by the phenol-disulphonic method as modified by Harper (9). One to five water extractions were analyzed.

Carbon dioxide evolution determinations were carried out by attaching the incubation flasks, which were set up in parallel, to absorption towers containing glass beads and known quantities of approximately 1/10 N. standard sodium hydroxide solution, which were changed and titrated each morning, using barium chloride in excess to precipitate the sodium carbonate formed. The excess sodium hydroxide was determined by titrating with standard hydrochloric acid and phenolphthalein as indicator, according to Scott (22).

The incubation flask was fitted with a rubber stopper through which two holes were bored into which glass tubes were inserted. One reached just below the stopper, permitting the



intake of air from the scrubbers, the other reached to the bottom of the flask which was covered with glass wool with soil above, thus allowing the removal of carbon dioxide from the bottom.

To prevent any back flow of carbon dioxide from the incubation flask a screw clamp was placed directly between it and the scrubbers. This screw was released immediately after the suction was applied and the solution began to rise in the absorption tower. The suction was applied for 45 minutes each day prior to titrating but the  $\text{CO}_2$  evolved had free access to wander into the alkaline absorbing medium at all times.

The air drawn through the system was first washed by passing it through scrubbers containing 35% sodium hydroxide solution, 10 N. sulphuric acid and finally through distilled water which was placed in the circuit to prevent the soil from drying out. At the other end of the system similar scrubbers were installed to wash any air passing back into the system when the suction was released.

The standard, hydrochloric acid and sodium hydroxide were made up in bulk and protected from the air. Carbon dioxide free water was used for washing the towers prior to titration.

Since carbon dioxide is a constituent of the air about us, one can readily see that it is a difficult determination. However, the method employed gave, as a rule, consistent results, and blanks run on the set-up agreed as well as could be expected.

One of the first problems that arose was whether the air should be drawn over or through the soil. To ensure the removal of all of the carbon dioxide, and to prevent anaerobic conditions from developing, it was decided to draw the air through the soil.



However, the method can be criticized in that it would necessarily leave the micro-organisms of the soil in an atmosphere, momentarily at least, devoid of carbon dioxide, which would possibly accelerate their activity. There would most likely be greater accumulation of carbon dioxide by the end of the twenty-four hour period, which in turn might inhibit the activity. It was therefore assumed that one factor would balance the other. If continuous aeration had been employed, it would have been difficult to control and would have, in all probability, resulted in more varied atmospheres for the organisms.



## RESULTS.

### NUMBER OF MICROORGANISMS IN UNSTERILIZED AND RECONTAMINATED STERILIZED ALBERTA SOILS

#### Bacteria

The numbers of bacteria in the Edmonton black and Fallis gray soils were much larger after two weeks incubation in the sterilized and recontaminated soil than in the original soil, and after ten weeks in the Gros Ventre brown soil, as indicated by Table 1 and Fig. 1 and 2.

The Edmonton sterilized recontaminated soil showed the greatest increase from this treatment of the three soils studied. As brought out by Fig. 1, there were fluctuations in numbers until the end of the eight weeks when a decided increase took place which was maintained until the end of the 14th week. There was then a drop in numbers but at the termination of the experiment at the end of the 22nd week there was still a large difference, the sterilized recontaminated having a count of 80 million as compared with 13 million in the unsterilized.

Fallis gray wooded sterilized and recontaminated soil compared with the unsterilized, gave much the same graphic picture, Fig. 2, as the corresponding treatments in the Edmonton black loam. The peak was reached at ten weeks from which point the numbers gradually decreased, until at the end of the 22nd week the sterilized recontaminated had a count of 54 million compared to 12 million for the unsterilized.

The Gros Ventre brown prairie soil, intermediate in fertility to the other two soils, presented a very different picture, Fig. 3.



The sterilized recontaminated soil counts fluctuated around the unsterilized soils counts and were not consistently greater.

### Fungi.

The numbers of fungi were much larger in all cases in the sterilized and recontaminated soil than in the unsterilized.

Edmonton black loam, sterilized recontaminated, and unsterilized showed the characteristic fluctuations in plate counts until the end of the third week, when the unsterilized soil counts were approximately 100,000 per gram for the duration of the experiment, Fig. 3. The sterilized recontaminated took an upward trend from the three week period until the maximum count was obtained at the end of six weeks, when the count was twenty-four times greater than for the unsterilized. The curve then gradually dropped until the end of the twenty-second week when a count of 600,000 was obtained for the sterilized recontaminated and 60,000 for the unsterilized.

Fig. 4, comparing Fallis gray wooded unsterilized and sterilized recontaminated soils, shows a decided increase in numbers of fungi for steam sterilized recontaminated from the beginning of the experiment until the end of the eighteenth week, when a rather sudden drop occurred until at the end of the twenty-second week the sterilized recontaminated had a count of 480,000 compared to 32,000 in the unsterilized. The high point was reached at the fourteenth week when the ratio was approximately 1:15 for unsterilized as against sterilized recontaminated.

The Gros Ventre fungi counts were much higher for the unsterilized and sterilized recontaminated soils than for the other two soils under investigation. Steam sterilization and re-



contamination caused a greater increase in the total count when compared to the unsterilized. In general the graphical picture No. 5 follows much the same trend. The unsterilized and sterilized recontaminated soil counts were much the same at the end of one week, gradually increasing in the case of the steam sterilized recontaminated soil until the end of the third week when a sudden increase took place. The maximum count of 12,800,000 was obtained at the end of the sixth week for the sterilized recontaminated compared to a count of 70,000 for the unsterilized. Then there was a general decrease in numbers until the end of the fourteenth week when a sudden decrease took place and at the twenty-second week the sterilized recontaminated gave a count of 900,000 compared to 60,000 in the unsterilized.

The counts of bacteria in the Edmonton black loam, and the Fallis gray wooded silt loam, were much higher than in the Gros Ventre brown prairie loam, which was directly the reverse of the fungi counts.

#### Protozoa.

The data presented in Table 2 does not give a true picture of the numbers of protozoa that are to be found in these soils, as dilutions were not made over 100,000 per gram, and protozoa were found in many cases at this dilution. Nevertheless, a few points of interest were observed.

Edmonton black loam, unsterilized, has a protozoan population greater than 100,000 per gram, under the conditions of the experiment after an incubation period of four weeks. The sterilized recontaminated soil appears to have a more fluctuating protozoan count as shown from the two week period to the end of the eighth week.



In comparing Fallis gray wooded sterilized recontaminated soil with the unsterilized, the protozoan population seems to favor the unsterilized soil as a medium for growth. Only once in the ten week period did the sterilized recontaminated exceed the count in the unsterilized soil. After the tenth week the protozoan population showed a tendency to increase. The counts in Fallis unsterilized and sterilized recontaminated soil showed greater variability than the other two soils.

The general tendency of the counts for unsterilized Gros Ventre brown prairie soil was to be lower than or equal to that of the sterilized recontaminated. In the unsterilized, protozoa were present at 10,000 but absent in the 100,000 dilution in the majority of cases; only in one case did the unsterilized have a count greater than 100,000 per gram.



Table 1. Numbers of microorganisms in unsterilized and reinoculated sterilized Alberta soils.

		Period of incubation after sterilizing soil and setting up experiment.										
	Right after ster.	Bacteria: Millions per Gram										
		1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	8 wks.	10 wks.	14 wks.	18 wks.	22 wks.
Edmonton black park	6.2	---	14.9	14.0	13.4	12.6	23.7	16.9	25.0	17.5	10.8	13.6
	---	---	63.2	40.7	21.2	16.7	69.1	50.4	164.0	164.0	110.6	81.6
Gros Ventre brown prairie	4.2	---	5.8	8.3	8.2	8.4	10.0	7.3	13.0	9.0	4.3	6.3
	---	---	14.0	11.4	3.2	2.7	13.6	8.3	28.0	12.4	8.1	6.5
Fallis gray wooded	2.9	---	11.2	10.8	8.1	7.9	11.0	7.8	12.0	15.4	10.4	12.0
	---	---	23.0	42.8	37.6	25.9	46.8	31.0	104.0	72.0	58.8	53.8
		Fungi: Thousands per Gram										
Edmonton black park	181	420	800	70	80	90	100	90	100	55	73	58
	---	570	1550	780	1800	2200	2400	1700	1500	1400	1100	620
Gros Ventre brown prairie	129	90	140	80	370	190	80	60	120	80	53	60
	---	140	800	1010	9900	9900	12800	7900	8900	6480	1130	900
Fallis gray wooded	198	80	100	50	10	40	70	30	20	78	38	32
	---	310	1020	670	900	800	1000	700	700	1150	1100	480



Table 2. Numbers of protozoa in unsterilized and recontaminated sterilized Alberta soils.  
Protozoa - (Thousands per gram).

October 10, 1939.		Period of incubation after sterilizing soil and setting up experiment.											
		Weeks.											
		Started	1	2	3	4	5	6	8	10	13	16	20
Edmonton	Unsterilized	10 <sup>13</sup> .	10 <sup>12</sup> .	10 <sup>24</sup> .	10 <sup>34</sup>	100 <sup>1</sup>	100 <sup>2</sup>	100 <sup>12</sup>	100 <sup>12</sup>	100 <sup>12</sup>	100 <sup>12</sup>	100 <sup>1</sup>	100 <sup>1</sup>
"	Sterilized recontaminated with original soil	--	10 <sup>12</sup>	10 <sup>1</sup>	1 <sup>1</sup>	100 <sup>1</sup>	10 <sup>1234</sup>	10 <sup>23</sup>	10 <sup>13</sup>	100 <sup>2</sup>	100 <sup>12</sup>	100 <sup>124</sup>	10 <sup>13</sup>
Gros Ventre	Unsterilized	10 <sup>123</sup>	1 <sup>1</sup>	10 <sup>1</sup>	1 <sup>2</sup>	100 <sup>1</sup>	10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>1</sup>	10 <sup>124</sup>	1 <sup>123</sup>	10 <sup>1</sup>	100 <sup>1</sup>
"	Sterilized recontaminated with original soil	--	1 <sup>3</sup>	10 <sup>13</sup>	10 <sup>123</sup>	100 <sup>12</sup>	10 <sup>23</sup>	100 <sup>1</sup>	100 <sup>1</sup>	10 <sup>124</sup>	100 <sup>2</sup>	100 <sup>12</sup>	100 <sup>2</sup>
Fallis	Unsterilized	10 <sup>13</sup>	10 <sup>12</sup>	10 <sup>1</sup>	10 <sup>14</sup>	1 <sup>123</sup>	1 <sup>24</sup>	100 <sup>2</sup>	100 <sup>12</sup>	10 <sup>12</sup>	100 <sup>1</sup>	100 <sup>1</sup>	10 <sup>12</sup>
"	Sterilized recontaminated with original soil	--	1 <sup>23</sup>	1 <sup>4</sup>	1 <sup>1</sup>	10 <sup>1</sup>	1 <sup>12</sup>	10 <sup>2</sup>	10 <sup>12</sup>	10 <sup>12</sup>	100 <sup>1</sup>	100 <sup>1</sup>	10 <sup>2</sup>

1 Inactive - variable size and shape.

2 Inactive - round, regular margin, granular, brownish.

3 Inactive - round, irregular margin, clean, except for dark spots.

4 Active - variable size and shape. Clean, except for dark spots. Probably ciliates.



Figure 1.

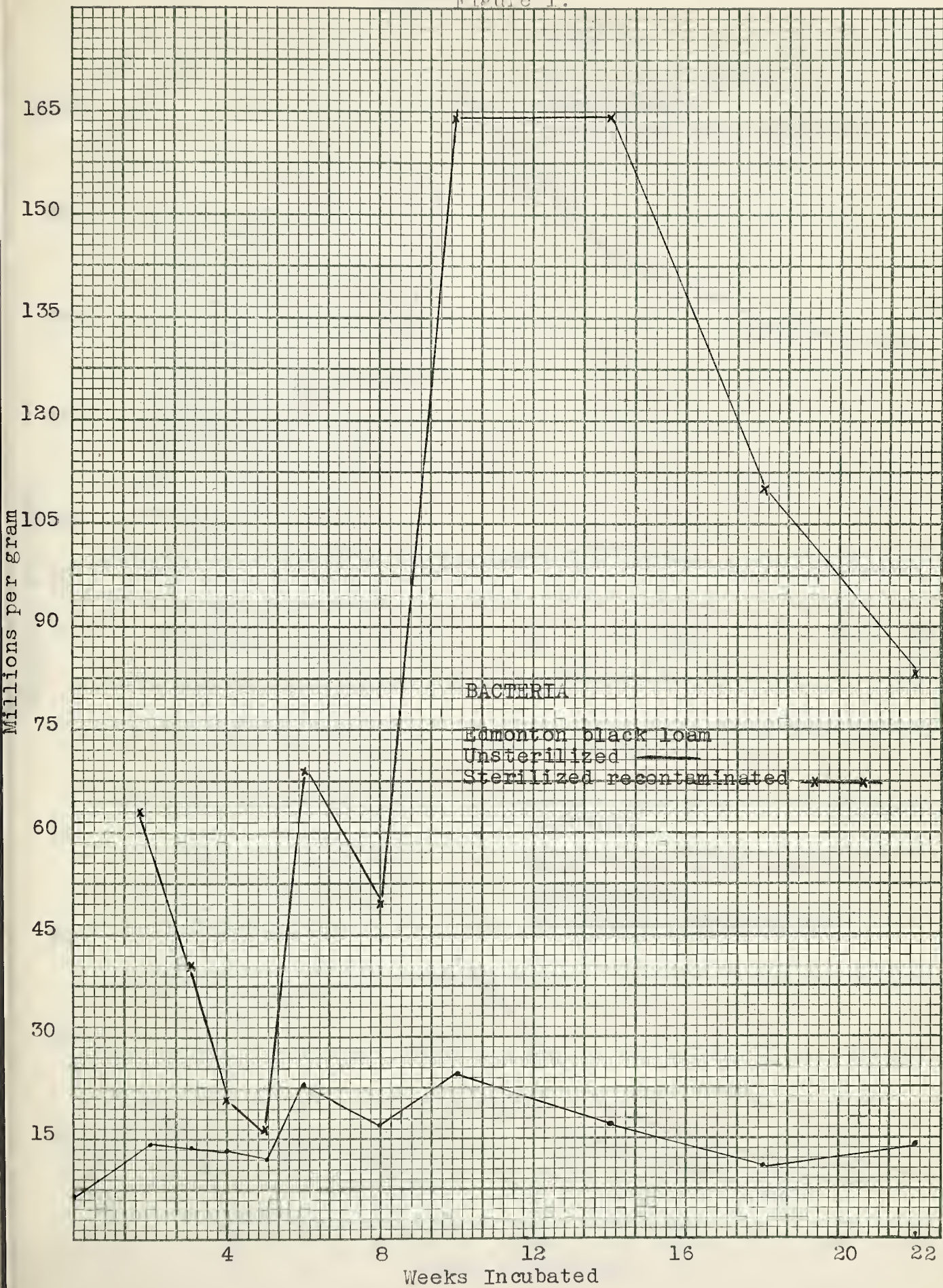




Figure 2





Figure 3.

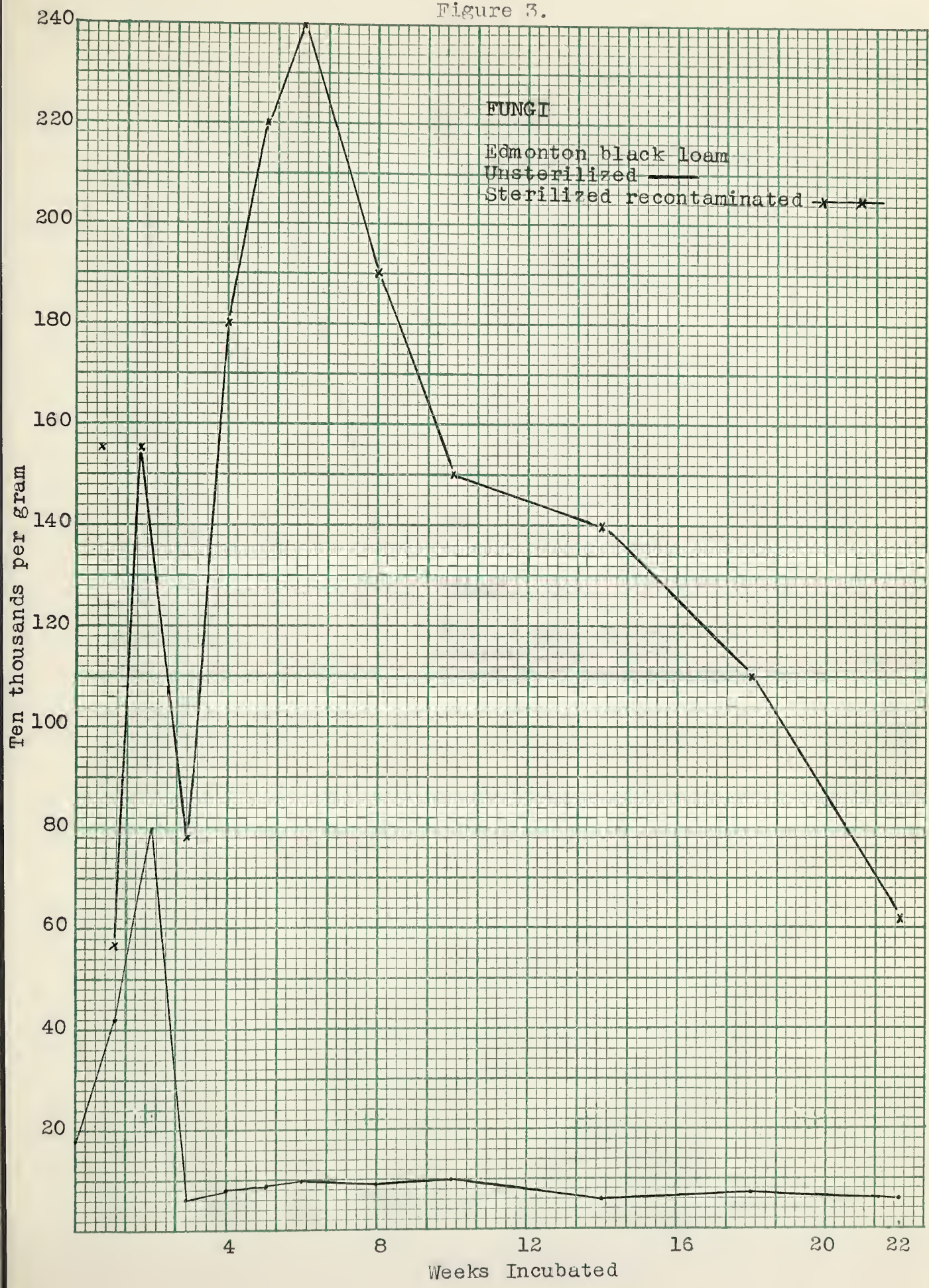




Figure 4.

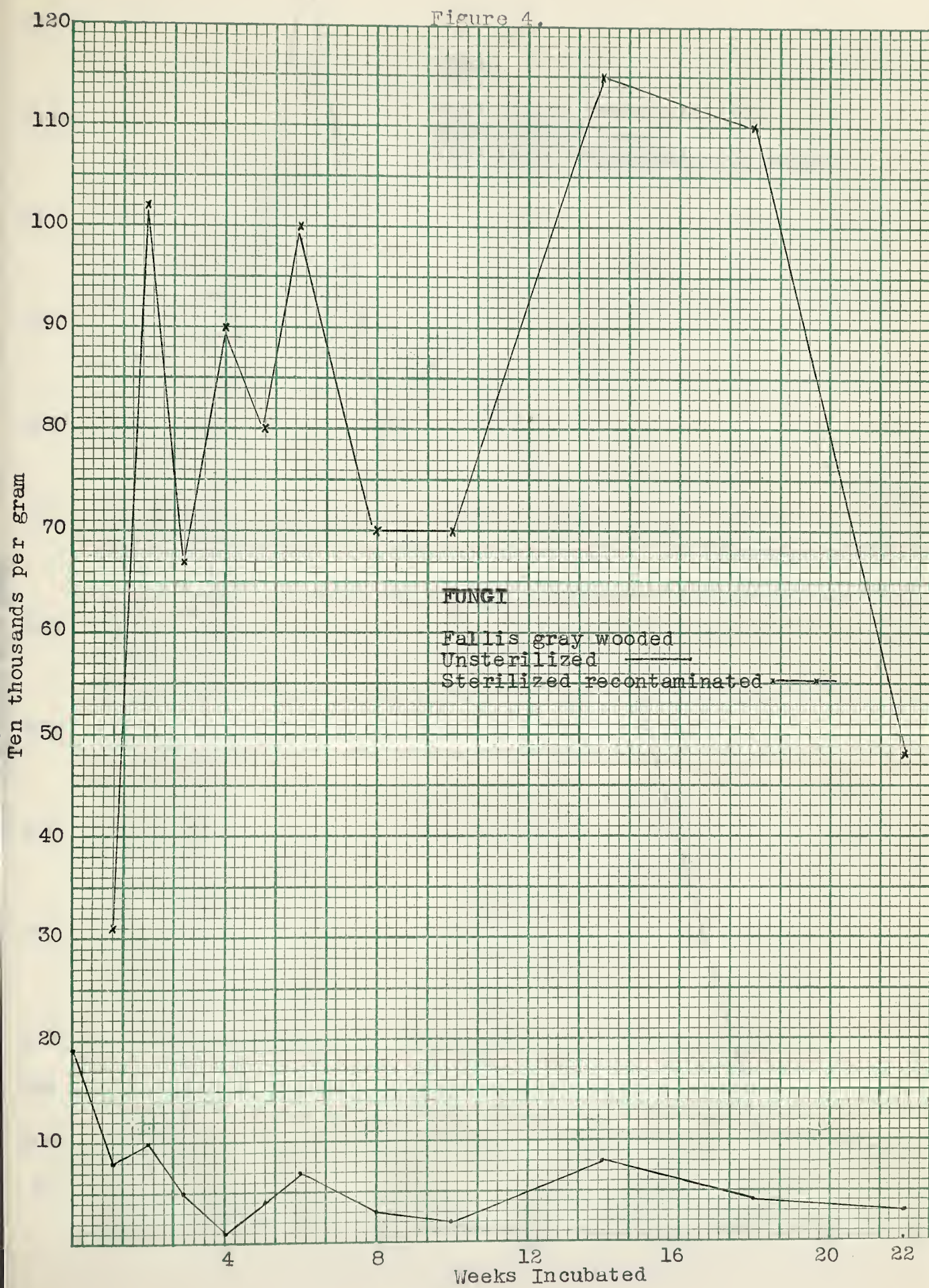
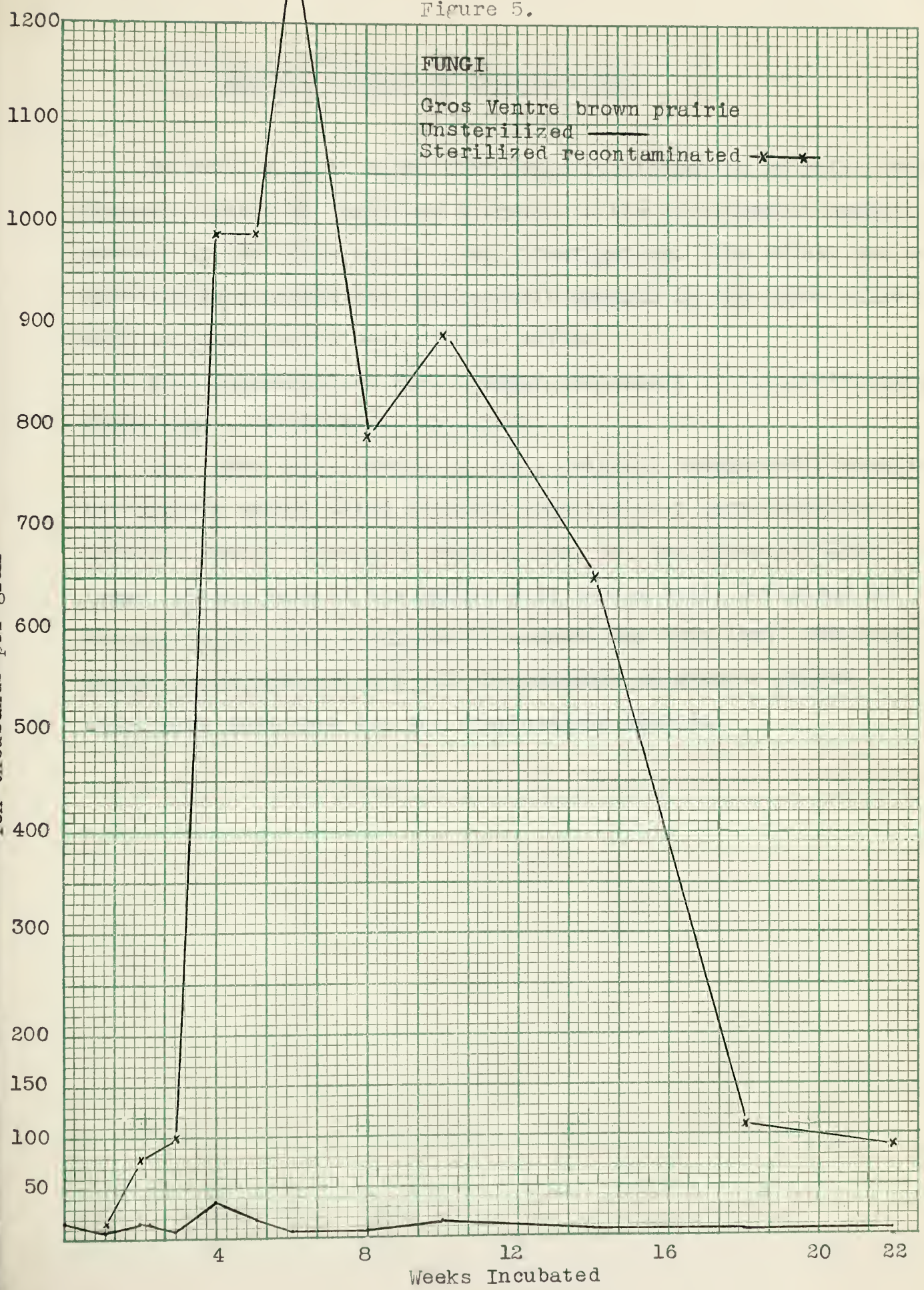




Figure 5.

Ten thousands per gram





IMMEDIATE EFFECTS OF STEAM STERILIZATION ON  
AMMONIA CONTENT OF ALBERTA SOILS.

In Table 3 it is shown that steam sterilization of soils under pressure caused an immediate increase in the displaceable ammonia content of the three soils under investigation. The effect was to approximately double the displaceable ammonia in the three soils when compared to the unsterilized soils.

The determinations were carried out singly at three different dates with a fairly close agreement.

The three soils varied in their original displaceable ammonia, the highest amount being obtained from the soil containing the greatest amount of organic matter, that is, the Edmonton black loam. In the case of the Fallis gray wooded soil, deficient in organic matter, the displaceable ammonia was very low. The Gros Ventre brown prairie soil was intermediate in organic matter which also held true for the displaceable ammonia.



Table 3. Immediate effect of steam sterilization on ammonia content of Alberta soils.

Ammonia nitrogen p.p.m. on water-free basis					
Soil	Treatment	Oct. 5/39	Nov.30/39	Feb.5/40	Average
Edmonton black park	Unsterilized	46	46	45	46
	Sterilized	83	82	75	80
Gros Ventre brown prairie	Unsterilized	26	22	19	22
	Sterilized	58	41	50	50
Fallis gray wooded	Unsterilized	5	6	13	8
	Sterilized	18	19	26	21



Description of Organisms Used in the Investigation

The following pure cultures were supplied by Dr. Henry and Mr. Ludwig, Department of Field Crops, Division of Plant Pathology, University of Alberta, Edmonton:

Fungus No. 3 - Unidentified, non-antagonistic, isolated from Edmonton black loam.

Fungus No. 19 - Aspergillus sp., non-antagonistic, isolated from Edmonton black loam.

Fungus No. 32 - Gliocladium sp. (~~Them~~), highly antagonistic, isolated from Edmonton black loam (only once).

Fungus No. 44 - Penicillium sp., non-antagonistic, isolated from Edmonton black loam.

Fungus No. 1001 - Trichoderma sp. (~~Them~~), moderately antagonistic, isolated from Edmonton black loam.

Fungus No. 1003 - Unidentified, moderately antagonistic, isolated from Edmonton black loam.

Fungus No. 1013 - Unidentified, moderately antagonistic, isolated from Edmonton black loam.

Ophiobolus No. 4 - A virulent strain of Ophiobolus graminis isolated from straw.

Pyronema confluens - Probably non-antagonistic, isolated from Edmonton black loam.



Ammonifying Power of Pure Cultures of Fungi,  
in Sterilized Edmonton Black Loam.

Two experiments to determine ammonifying power of pure cultures of fungi were conducted in Edmonton soil at different times. Fungus No. 32 was used in both experiments. The data obtained are presented in Table 4, and have been graphically represented in Figure No. 6 and No. 7. The curves for the highly antagonistic fungus No. 32, Gliocladium sp., follow the same trends in both experiments except for minor variations, probably due to varying conditions at the different times the experiments were carried out. From the organisms studied there is no very close relationship between antagonism or non-antagonism and ammonifying power.

A highly antagonistic fungus No. 32, Gliocladium sp., and a moderately antagonistic fungus No. 1013 are both strong ammonifiers as well as the non-antagonistic fungi No. 44, Penicillium sp., and Pyronema confluens. Two fungi were low ammonifiers, one of which, No. 1003, is moderately antagonistic, the other, No. 19, Aspergillus sp., is non-antagonistic. Fungus No. 1001, Trichoderma sp., was a low ammonifier which had the property of being moderately antagonistic. Malowany (14) found the non-antagonistic fungus No. 3 to be a low ammonifier.

An examination of the Figures shows that the high and intermediate ammonifiers reached their maximum at the end of the tenth week. The low ammonifiers produced very little ammonia until the end of the tenth week, when their activity became greater, reaching the maximum ammonifying power by the eighteenth week under the conditions of the experiment.



Ammonification in Fallis Gray Wooded Soil, Sterilized  
and Recontaminated with Pure Cultures of Fungi

The ammonifying powers of pure cultures of fungi in Fallis soil are shown in Table 4. An examination of the results obtained indicates that the ammonifying power of the cultures under investigation are very low. There is a slight rise as the experiment progresses, and variations between cultures are slight. Ophiobolus graminis and the highly antagonistic fungus No. 32, Gliocladium sp., both high ammonifiers in Edmonton black loam, do not show this characteristic when compared to the non-antagonistic fungi No. 3 and Aspergillus sp., no. 19, which were low ammonifiers in the Edmonton black loam.



Table 4. Ammonification in Edmonton black soil and Fallis gray wooded soil, sterilized and recontaminated with pure cultures of fungi.

(Average p.p.m. ammonia nitrogen on water-free basis)

Period of incubation after sterilizing soil and setting up experiment															
Right after ster.	Weeks														
	1	2	4	5	6	8	10	12	14	16	18	20	21		
<u>Started Jan. 24, 1938</u>															
Edmonton soil															
Fungus No. 3, Unidentified Non-antagonistic	97	76	125	200	116	96		140	160	159	209	213	184		
<u>Started Oct. 14, 1938</u>															
Edmonton soil															
Fungus No. 32, Gliocladium sp. Highly antagonistic	91	167	237	245	264	226	296	271	281	256	236	271			
Fungus No. 44, Penicillium sp. Non-antagonistic	102	144	152	151	201	185	235	258	251	259	292	279			
Fungus No. 19, Aspergillus sp. Non-antagonistic	86	85	81	105	107	95	97	112	105	145	169	92			
Fungus No. 1003, Unidentified Moderately antagonistic	80	82	90	91	109	107	107	128	148	173	187	144			



Table 4 (continued)

(Average p.p.m. ammonia nitrogen on water-free basis)

Period of incubation after sterilizing soil and setting up experiment														
Right after ster.	Weeks													
	1	2	3	4	5	6	7	10	12	14	16	18	20	22
<u>Edmonton soil</u>														
Started March 1, 1939														
Fungus No. 32, Gliocladium sp. Highly antagonistic	136	135	-	222	-	249	266	302	274	286	277	328	314	303
Fungus No. 1013, Unidentified Moderately antagonistic	132	123	-	186	-	192	239	250	247	273	259	256	250	250
Fungus No. 1001, Trichoderma sp. Moderately antagonistic	173	158	-	160	-	163	177	198	177	176	122	191	184	182
Fungus. Pyronema confluens Non-antagonistic	198	186	-	195	-	227	226	253	217	226	202	237	241	241
<u>Fallis soil</u>														
Started Nov. 17, 1939														
Ophiobolus graminis, No.4 Root rotting fungus	31	26	31	43	51	55	44	53	45	52	62			
Fungus No. 3, Unidentified Non-antagonistic	31	29	31	41	49	50	44	66	53	56	75			
Fungus No. 32, Gliocladium sp. Highly antagonistic	31	32	29	49	52	65	44	59	55	63	72			
Fungus No. 19, Aspergillus sp. Non-antagonistic	31	26	35	34	35	41	31	69	36	53	65			



Figure 6.

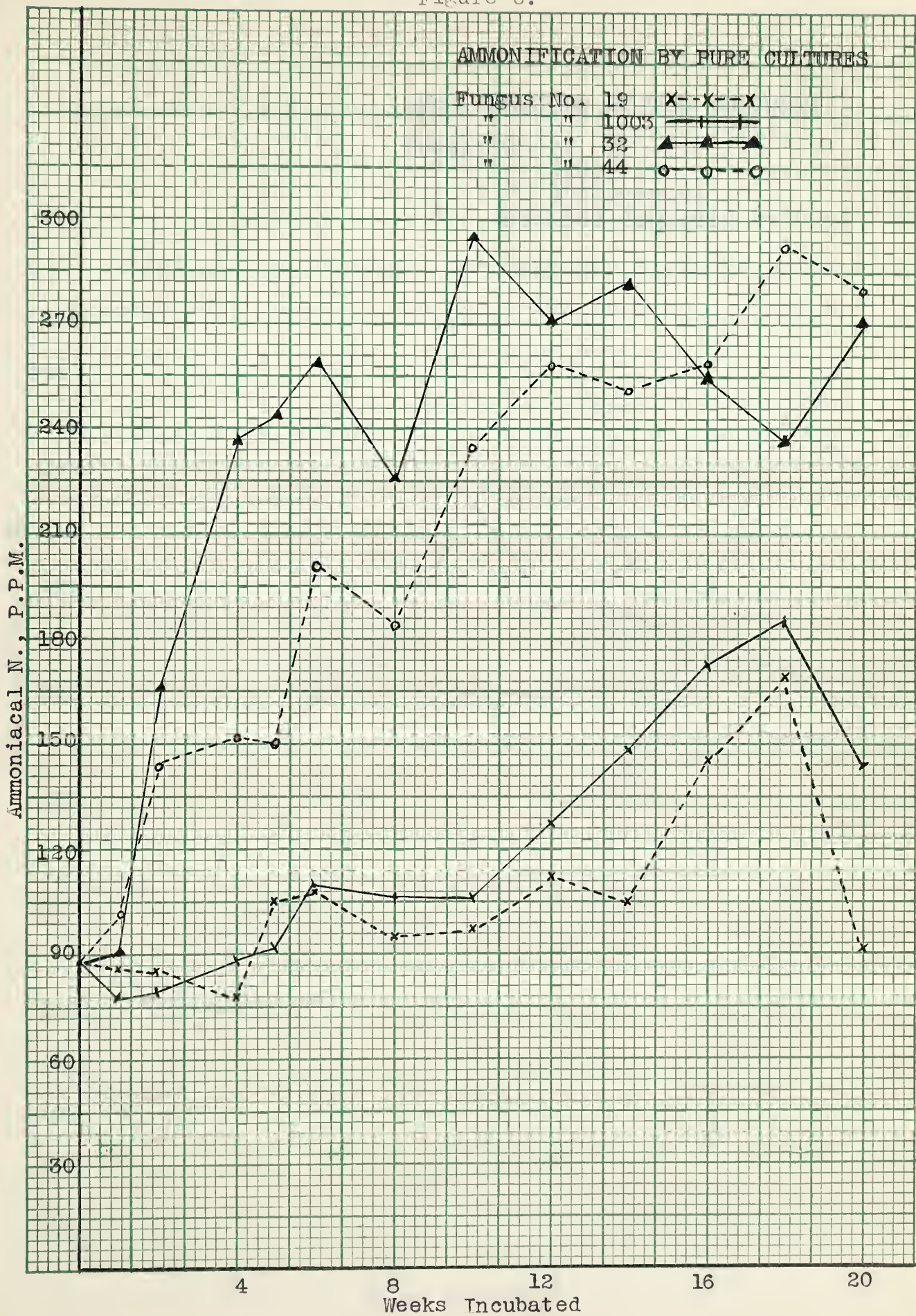
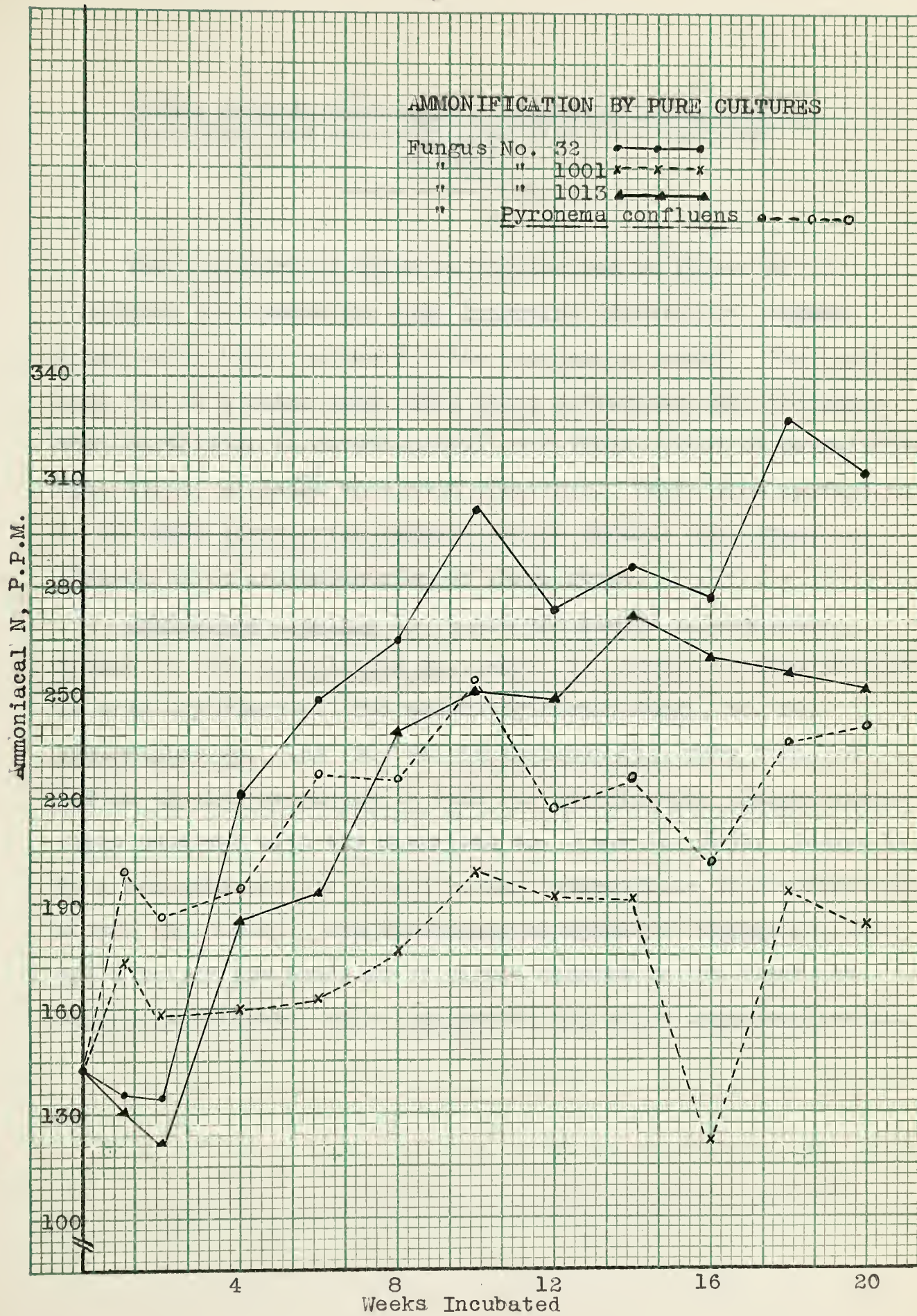
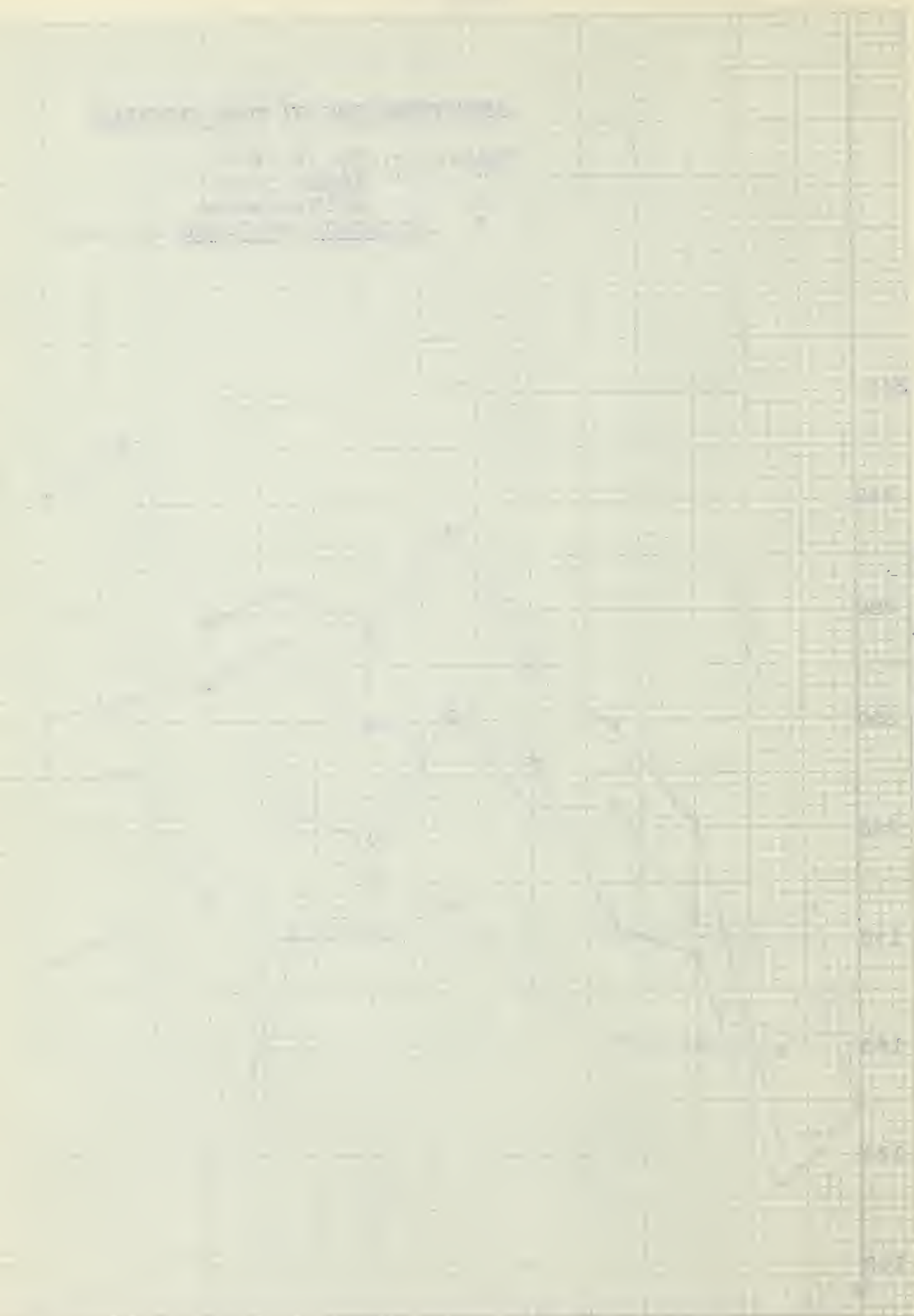




Figure 7.





Carbon Dioxide Production in Edmonton Black Soil  
Sterilized and Recontaminated with Pure Cultures  
of Fungi

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Table 5, part of which is represented in Figure No. 8, indicates that there are some important differences between fungi as to their carbon dioxide production powers. The duplicate run at a later date shows these variations, and indicates that variations in environmental conditions affect their relative positions, <sup>or</sup> at least with some organisms. There also appears to be no close correlation between carbon dioxide production and antagonism or non-antagonism of these fungi.

Ophiobolus graminis No. 4 and the highly antagonistic fungus No. 32, Gliocladium sp., were high carbon dioxide producers in both experiments. Two non-antagonistic fungi, No. 19, Aspergillus sp., and No. 44, Penicillium sp., were both low carbon dioxide producers in both the experiments. Slight variations occurred with the organisms No. 3 and No. 1003. Fungus No. 3, a non-antagonistic organism, was intermediate in the first and second experiment. The moderately antagonistic fungus No. 1003 was a fairly low producer of carbon dioxide in the first and second experiments, when compared to the other organisms tested.



Table 5. Mg. of carbon dioxide produced by pure cultures of fungi in Edmonton sterilized black loam.

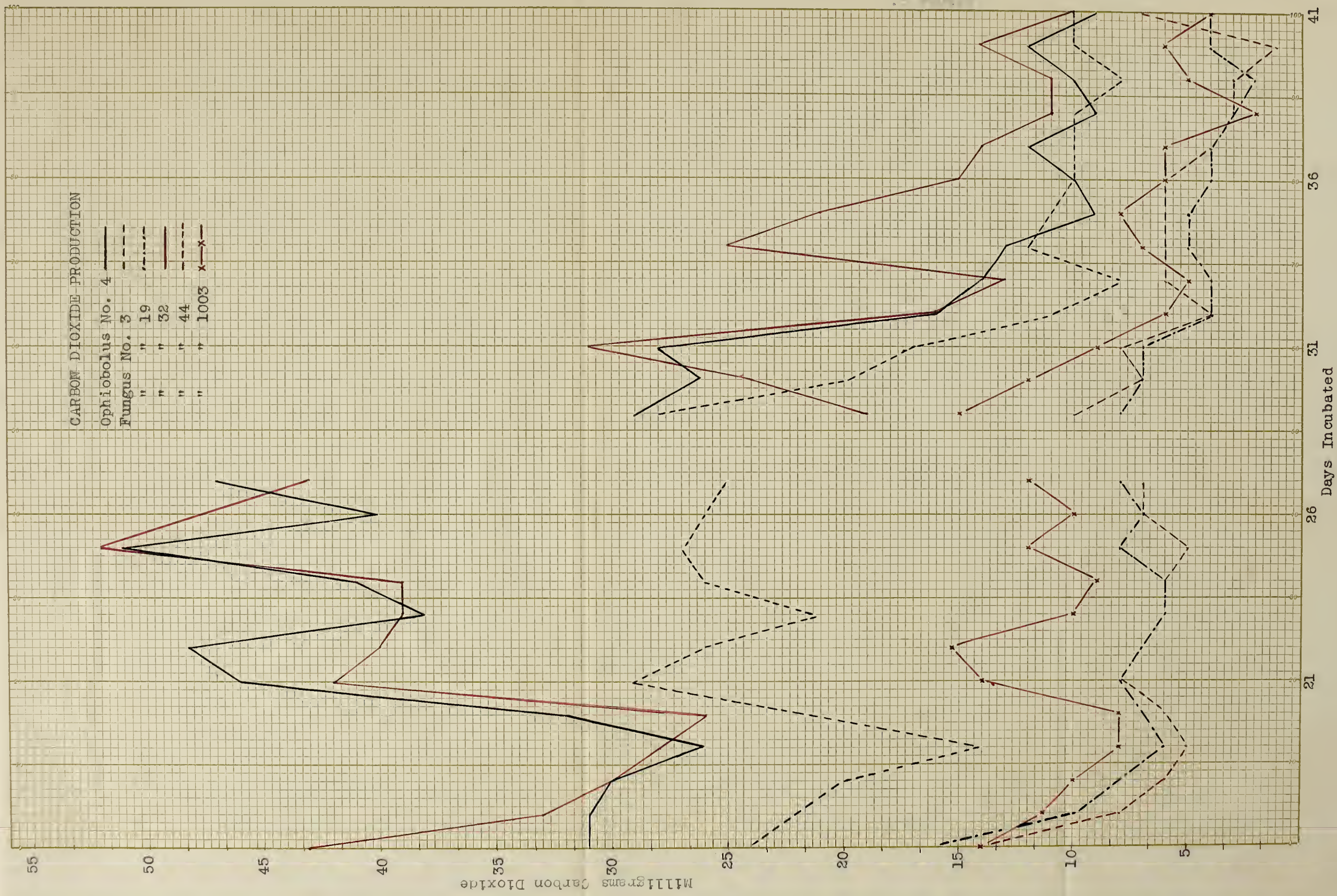
Jan. 20, 1939.	Fungi Numbers						Sterilized Soil
	Days	Ophiobolus	3	19	32	44	
15	26	24	37	68	23	21	16
16	31	24	16	43	14	14	4
17	31	22	10	33	8	11	3
18	30	20	8	30	6	10	3
19	26	14	6	28	5	8	3
20	32	22	7	26	6	8	5
21	46	29	8	42	8	14	3
22	48	26	-	40	7	12	7
23	38	21	6	39	6	10	10
24	41	26	6	39	6	9	21
25	51	27	8	52	5	12	32
26	40	8	7	13	7	10	44
27	47	25	8	43	7	12	61
28	-	-	-	-	-	-	-
29	29	28	8	19	10	15	4
30	26	20	7	24	7	12	4
31	28	17	7	31	8	9	4
32	16	11	4	16	4	6	4
33	12	8	4	13	6	5	5
34	20	12	5	25	6	7	8
35	16	11	5	21	6	8	17
36	14	10	4	15	6	6	24
37	13	10	4	14	4	6	32
38	9	6	3	11	3	2	21
39	10	8	2	11	3	5	16
40	12	10	4	14	1	6	39
41	9	10	4	10	7	4	29
42	12	11	5	7	6	6	29

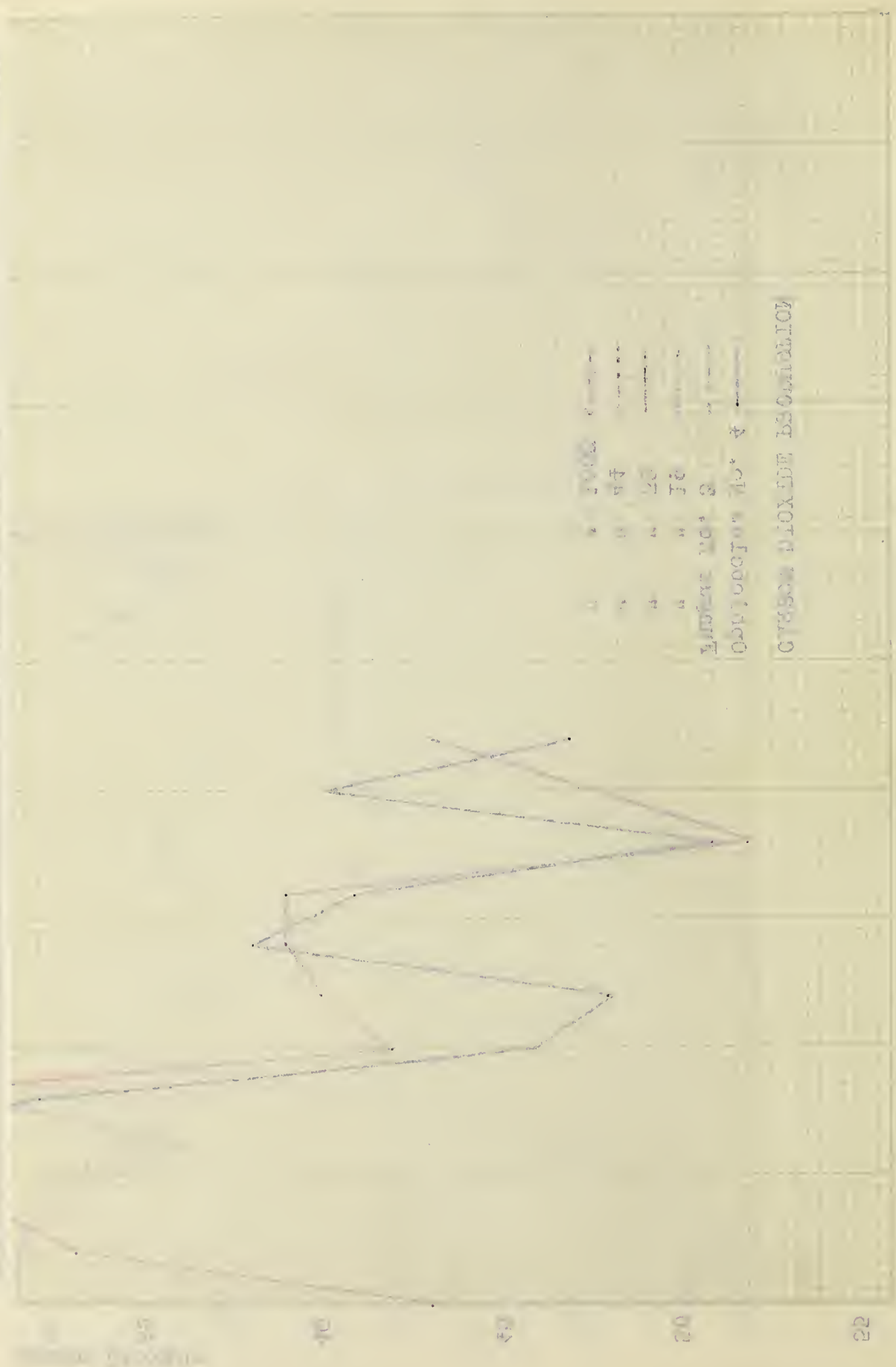


(Table 5 continued)

Mar. 11, 1939.		Fungi Numbers					Sterilized	
Days	Ophiobolus	3	19	32	44	1003	Soil	
15	25	9	18	23	24	24	5	
16	29	8	16	27	16	22	6	
17	31	7	13	25	--	23	10	
18	30	5	12	23	10	25	18	
19	34	6	11	27	8	27	23	
20	35	6	10	29	7	26	23	
21	38	5	10	31	7	26	26	
22	36	6	8	31	6	23	27	
23	33	4	6	28	4	19	24	
24	34	5	6	33	4	18	29	
25	29	4	5	31	5	17	23	
26	27	3	3	29	4	13	23	
27	23	4	3	21	2	10	16	
28	27	6	4	32	4	14	23	
29	43	27	13	53	9	26	8	
30	17	36	8	36	5	16	4	
31	25	7	4	29	4	10	3	
32	23	8	4	39	4	9	3	
33	23	10	3	26	4	10	3	
34	25	8	5	36	5	10	4	
35	19	9	4	32	3	11	4	
36	25	8	5	29	4	7	3	
37	18	7	3	20	2	7	4	
38	14	6	2	21	2	6	6	
39	13	5	2	16	2	5	7	
40	17	6	4	20	3	6	11	
41	15	6	3	16	3	6	13	
42	14	4	3	18	3	7	17	







L ———  
 U - - -  
 D .....  
 J - . - .  
 Legend: L, U, D, J

GRAPH OF THE FUNCTION

Carbon Dioxide Production in Unsterilized and Steam  
Sterilized Recontaminated with Original Soil

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More carbon dioxide was produced in the sterilized Edmonton black loam and Gros Ventre brown prairie loam recontaminated with original soil than in the unsterilized soil, as shown in Table 6 and Figures 9 to 11. Insignificant differences were obtained with the corresponding treatments in Fallis gray wooded soil.

Carbon dioxide production was increased approximately 100 percent through the treatment of steam sterilization and recontamination, and was maintained for a period of ten days, when production gradually decreased, but the steam sterilized recontaminated production was greater than in the unsterilized for the Edmonton and Gros Ventre soils for the duration of the experiment. There was greater evolution of carbon dioxide from the Edmonton black loam than from the Gros Ventre brown prairie loam, when comparisons are made of the similar treatments.

Fallis gray wooded soil was an extremely poor producer of carbon dioxide when compared to the Edmonton and Gros Ventre soils. The steam sterilization and recontamination compared to the unsterilized had very little effect. The daily variations were not great enough to indicate any significant effects from the treatment.

A duplicate experiment was conducted at a later date, the same general observations being noted. The Edmonton black loam and Gros Ventre brown prairie loam, sterilized and recontaminated, did not give the pronounced activity obtained in the first experiment. Carbon dioxide production dropped in the



second experiment at the end of two weeks to a point where very little effect could be measured from the treatment as compared to the unsterilized soils.



Table 6. Mg. of carbon dioxide produced in unsterilized and sterilized recontaminated, Edmonton, Gros Ventre and Fallis soils.

Days	Edmonton			Gros Ventre			Fallis			Fungus No. 32
	Unsterilized	Sterilized Recontam- inated		Unsterilized	Sterilized Recontam- inated		Unsterilized	Sterilized Recontam- inated		
11	11.0	24.4	68.5	43.1	73.1	15.3	29.4			
12	21.4	41.0	17.1	35.5	7.0	12.5	16.5			
13	16.2	34.9	12.8	31.2	5.0	9.8	12.5			
14	18.7	41.6	13.8	30.9	5.2	10.4	13.2			
15	19.0	38.2	15.9	32.7	6.7	11.0	13.5			
16	20.2	38.8	18.0	33.0	8.3	11.3	15.3			
17	22.3	48.0	19.6	34.9	8.0	11.0	16.5			
18	20.5	37.9	15.9	30.0	6.7	9.8	15.3			
19	20.8	38.8	18.7	31.2	7.7	10.1	13.2			
20	18.7	31.2	14.4	26.0	7.3	8.9	12.8			
21	21.7	41.6	19.0	31.8	8.6	10.7	14.4			
22	17.4	30.0	13.8	22.3	6.1	7.7	12.2			
23	18.0	30.3	17.4	26.0	6.4	8.3	12.8			
24	19.3	27.4	15.6	24.5	6.4	8.0	14.4			
25	17.4	33.3	18.0	21.0	6.1	7.7	12.2			
26	17.4	31.2	18.4	22.9	5.8	7.3	11.9			
27	17.1	29.4	16.5	21.4	6.1	7.3	11.6			
28	18.0	30.0	17.4	21.4	6.4	7.3	12.8			
29	19.3	26.6	18.0	21.7	6.1	7.3	12.2			
30	16.5	25.4	18.0	22.0	6.7	6.7	15.9			
31	15.6	22.3	16.5	18.0	5.5	6.4	10.1			
32	13.6	24.8	14.4	15.9	4.6	8.3	5.5			



(Table 6 continued)

Oct. 7, 1939.	Edmonton		Gros Ventre		Fallis		Fungus No. 32
	Unsterilized	Sterilized Recontam- inated	Unsterilized	Sterilized Recontam- inated	Unsterilized	Sterilized Recontam- inated	
32	12.3	21.1	12.6	15.9	3.6	4.6	14.7
33	13.2	19.9	11.6	16.5	3.7	5.5	10.1
34	14.1	20.5	13.2	20.2	4.6	5.2	9.2
35	15.0	21.4	12.5	20.2	4.3	6.4	9.8
36	13.6	18.3	12.0	20.5	4.4	5.4	8.5
37	16.2	21.1	14.1	23.0	4.9	6.5	8.3
38	18.1	---	14.4	24.2	4.9	5.5	11.0
39	19.6	---	14.7	19.0	4.3	6.1	10.7
40	16.6	---	14.1	24.2	4.6	5.8	9.2
41	16.5	---	13.2	20.2	4.0	4.9	8.3
42	14.1	---	12.5	18.6	3.4	3.1	6.1
43	17.1	---	13.8	23.2	1.5	5.8	6.7
44	16.8	---	15.6	22.7	7.7	5.8	8.6
45	15.0	---	12.2	17.8	3.8	5.2	7.7
46	12.8	---	11.9	16.8	3.4	4.3	6.5
47	15.3	---	14.4	18.4	4.3	4.6	7.0
48	15.0	---	14.4	19.8	4.3	4.9	7.4
49	14.4	---	13.8	18.4	4.0	4.6	6.4
50	14.4	---	13.5	19.0	4.0	4.6	6.7



(Table 6 continued)

Jan. 15, 1940.	Edmonton		Gros Ventre		Fallis		Fungus No. 32
	Unsterilized	Sterilized Recontam- inated	Unsterilized	Sterilized Recontam- inated	Unsterilized	Sterilized Recontam- inated	
10	29.1	48.7	13.5	21.4	18.7	26.6	33.4
11	22.3	37.3	12.2	23.9	13.8	22.3	23.9
12	21.1	39.8	10.7	23.0	18.4	22.6	27.2
13	24.5	34.0	13.8	25.1	13.2	25.4	39.2
14	25.4	38.6	15.6	23.0	14.4	23.0	42.2
15	18.4	18.4	11.3	16.0	9.8	14.1	32.4
16	19.0	21.4	12.2	19.3	11.0	17.1	41.3
17	21.7	20.8	15.3	20.5	13.2	16.5	45.0
18	16.5	17.7	12.2	16.5	11.0	13.2	39.2
19	16.5	20.5	12.9	18.4	11.0	13.2	34.0
20	17.2	15.0	13.5	19.0	10.7	13.2	32.7
21	10.1	17.4	13.5	20.5	12.2	13.5	29.4
22	12.5	17.2	14.7	17.7	11.0	11.9	24.8
23	17.4	20.2	13.8	18.0	11.6	12.3	20.2
24	21.4	22.0	18.6	24.8	13.8	15.6	20.8
25	15.3	21.7	13.7	19.0	11.0	11.3	19.0
26	8.9	17.5	12.5	18.1	12.5	10.4	13.2
27	15.3	18.4	14.4	17.4	13.1	11.3	12.8
28	8.6	15.0	8.6	11.0	7.0	6.7	10.1
29	13.8	17.8	12.2	14.4	10.2	8.9	12.6
30	13.5	17.7	13.5	12.3	11.6	10.4	12.9
31	10.1	13.5	9.8	12.0	8.9	6.9	9.2
32	10.1	12.3	9.2	11.3	9.8	7.4	8.6



(Table 6 continued)

Jan. 15, 1940.	Edmonton		Gros Ventre		Fallis		Fungus No. 32
	Unsterilized	Sterilized Recontam- inated	Unsterilized	Sterilized Recontam- inated	Unsterilized	Sterilized Recontam- inated	
32	13.8	29.1	9.8	11.0	5.5	8.3	18.4
33	13.8	15.9	9.8	11.3	7.4	6.7	13.8
34	10.4	12.5	8.3	11.0	6.7	6.4	10.4
35	13.7	15.0	9.8	12.3	8.3	8.6	14.4
36	13.2	18.1	11.0	12.3	8.9	8.3	14.1
37	15.6	17.4	11.6	14.7	9.5	10.1	14.4
38	12.3	14.4	8.3	10.4	6.7	6.1	9.2
39	11.3	12.9	8.3	9.5	6.7	7.3	9.8
40	8.9	9.5	6.7	8.6	4.6	6.1	6.1
41	9.8	9.8	7.4	8.6	5.5	6.4	8.9
42	11.0	11.6	8.9	8.0	7.0	7.0	8.3
43	10.1	11.3	7.7	8.3	6.1	6.4	6.8
44	10.1	11.3	7.3	8.5	6.1	5.8	6.8
45	8.3	9.8	6.7	7.7	5.8	5.2	6.4
46	9.2	11.6	8.0	8.6	6.1	6.1	7.4



Figure 9.

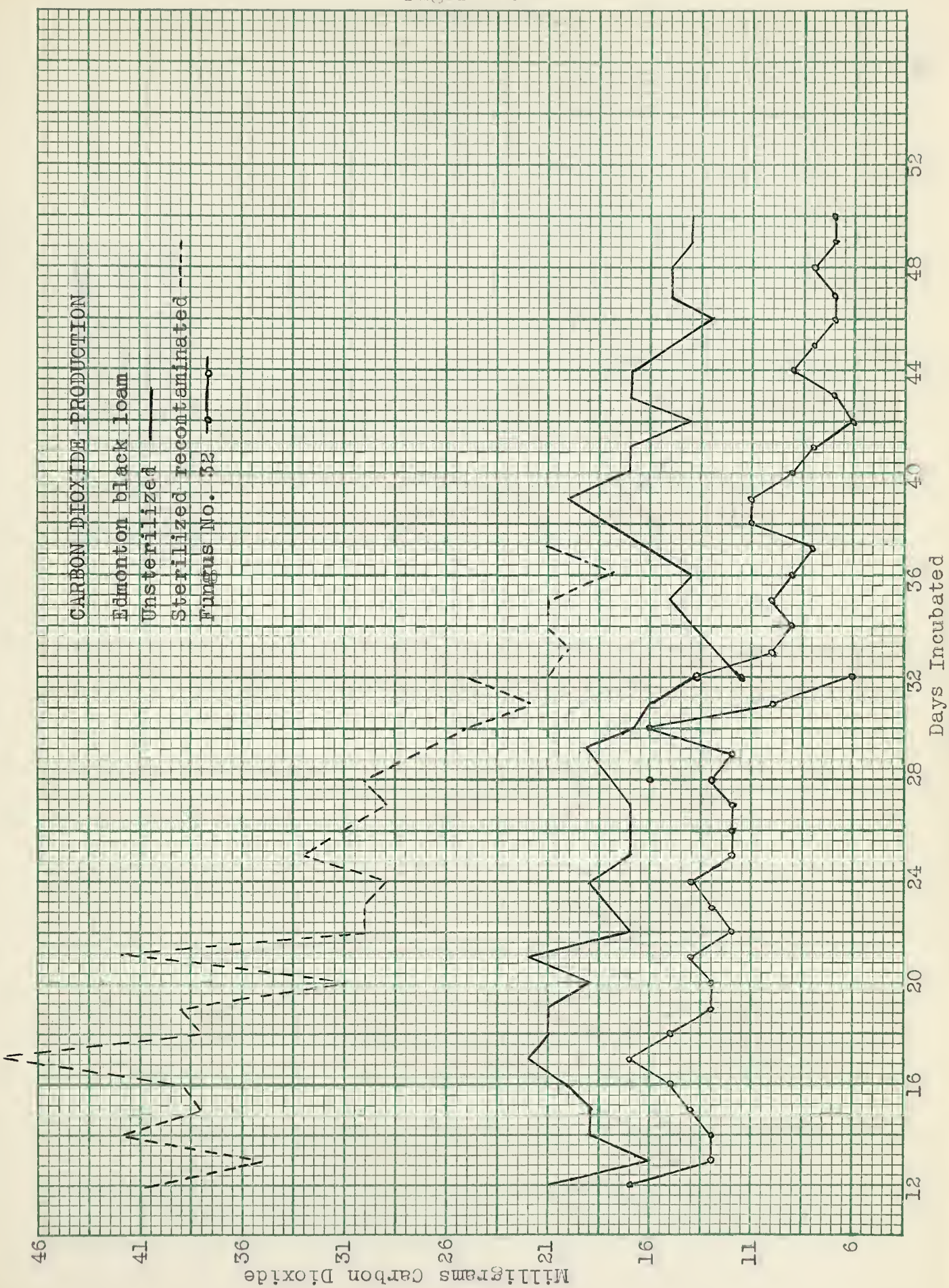




Figure 10.

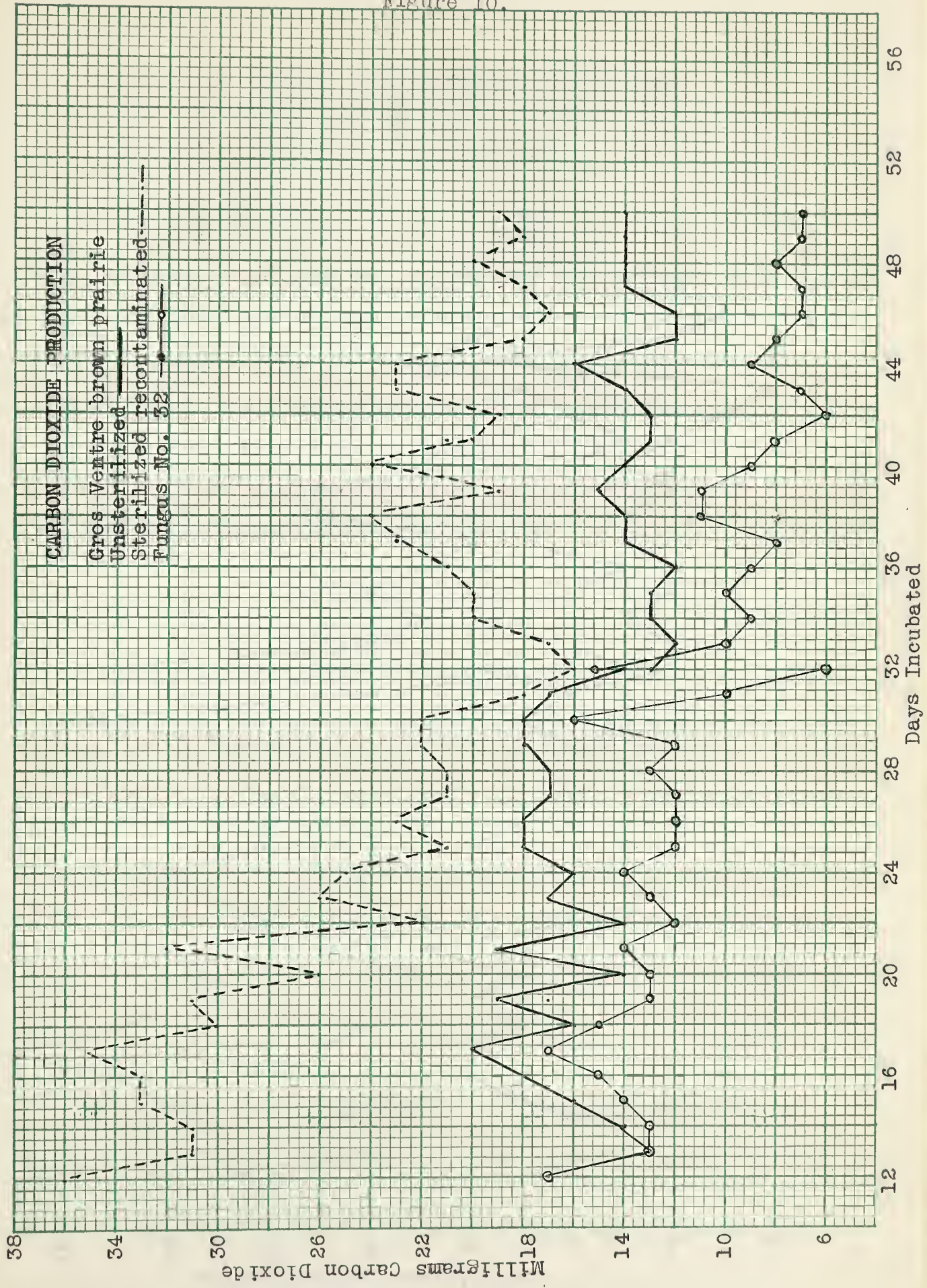
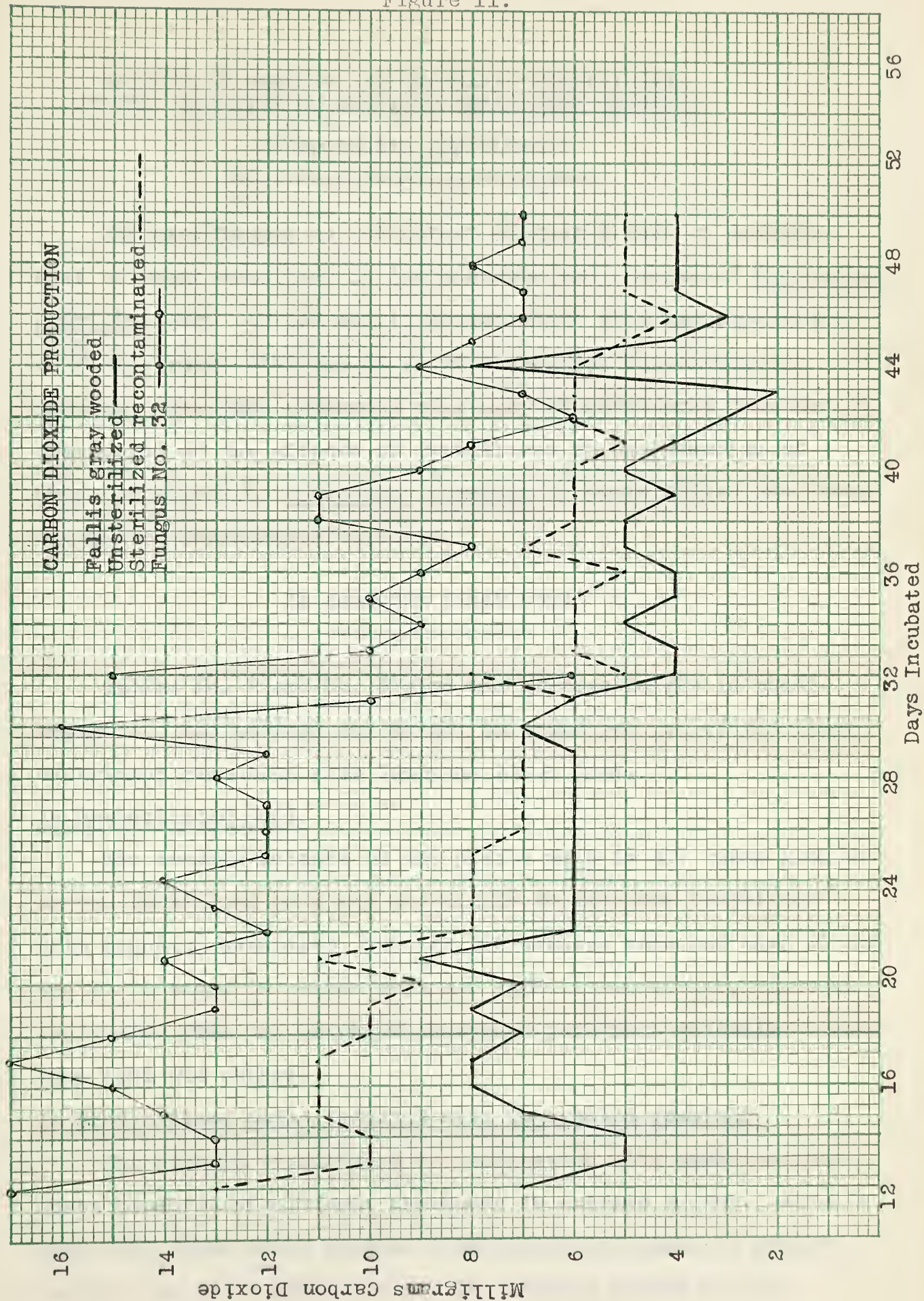




Figure 11.





# EFFECTS OF ORGANIC AND INORGANIC FERTILIZERS ON THE REACTION OF WHEAT TO OPHIOBOLUS GRAMINIS

## Co-operative Experiments

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The experiments on the effect of fertilizers on development of wheat seedlings in the presence and absence of Ophiobolus graminis, were carried out in co-operation with the Department of Field Crops, Division of Plant Pathology, University of Alberta. The data presented will only be considered from the standpoint of the effects of fertilizers on the reaction of wheat seedlings to Ophiobolus graminis in unsterilized and steam sterilized recontaminated soils.

## In Edmonton Black Loam

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Tables No. 7 and No. 8 give the average height in centimetres and the average dry weight in grams per pot of the first experiment conducted with Edmonton black loam.

### In Unsterilized Soil

The average heights of the plants were in all cases greater in the fertilized pots and especially in the pots fertilized with ammonium phosphate, but the differences were not significant. The differences in weight were not significant either, although the yields from the ammonium phosphate pots were generally higher than from the others.

### In Unsterilized Soil Infested with Ophiobolus graminis

The organic fertilizers in combination with ammonium phosphate produced significant increases in average height. Alfalfa alone and straw alone produce significant increases in average height, but not sawdust alone. The average height and the



average dry weights were in all cases greater in the fertilized plots, but the increases in weights were not significant.

Disease severity was reduced by all fertilizers but most by the organic fertilizers. The combinations of straw and ammonium phosphate, and sawdust and ammonium phosphate, gave the least disease and were approximately equal in their effect.

#### In Sterilized Soil Recontaminated with Original Soil

The average height was not increased significantly in any case, though there were some increases in the cases of the straw and sawdust alone and in combination with ammonium phosphate. The average weights were generally lower in the cases of the fertilized pots, and significantly lower in the case of the pots fertilized with alfalfa in combination with ammonium phosphate.

#### In Sterilized Soil Recontaminated with the Original Soil and Infested with *Ophiobolus Graminis*

The average height was increased to some extent, and probably significantly, by straw and sawdust, alone and in combination with ammonium phosphate. The effects of ammonium phosphate and alfalfa, separately and in combination, were not significant. The fertilizers gave no significant increases in weight, but the straw and sawdust separately and in combination with ammonium phosphate gave the highest average yields.

#### In Fallis Gray Wooded Silt Loam, First Pot Culture Experiment

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Tables No. 9 and No. 10 give the average height in cm. and the average dry weight in grams per pot of the experiment conducted on Fallis gray wooded soil.



Infesting the soil with Ophiobolus graminis produced a very large increase in the percentage of disease, and very large and significant decreases in plant height and dry weight.

All fertilizers increased the average heights of the plants but these increases were not significant in the cases of ammonium phosphate, sawdust and sawdust plus ammonium phosphate. All fertilizers apparently gave some decrease in the percentage of disease, but some of the differences were probably not significant. Ammonium phosphate itself had little effect on the disease. The greatest decreases in disease severity were given by straw and straw plus ammonium phosphate.

#### In Unsterilized Soil

The heights were in all cases greater in the fertilized pots, but the increases were not significant. The straw plus ammonium phosphate, alfalfa plus ammonium phosphate, and the alfalfa produced large and significant increases in dry weight, and the sawdust plus ammonium phosphate produced a significant increase. Sawdust alone and ammonium phosphate alone produced a significant decrease.

#### In Unsterilized Soil Infested with Ophiobolus graminis

Straw plus ammonium phosphate gave the largest increases in height and dry weight. Significant increases in dry weight were also given by straw and alfalfa.

#### In Sterilized Soil Recontaminated with Original Soil

The fertilizers did not produce any significant increase in average height, but significant increases in dry weight were given by straw plus ammonium phosphate, alfalfa, and alfalfa plus ammonium phosphate. The greatest increase was given by



straw plus ammonium phosphate and a significant decrease by sawdust.

In Sterilized Soil Recontaminated with Original Soil and Infested with *Ophiobolus Graminis*

The heights were greater in the fertilized pots in all cases, but the increases were significant only in the cases of straw, straw plus ammonium phosphate and sawdust. All fertilizers gave significant increases in dry weight; the largest increases were given by straw plus ammonium phosphate and the smallest by ammonium phosphate. This is the only case in this experiment in which ammonium phosphate itself produced a significant increase in growth.





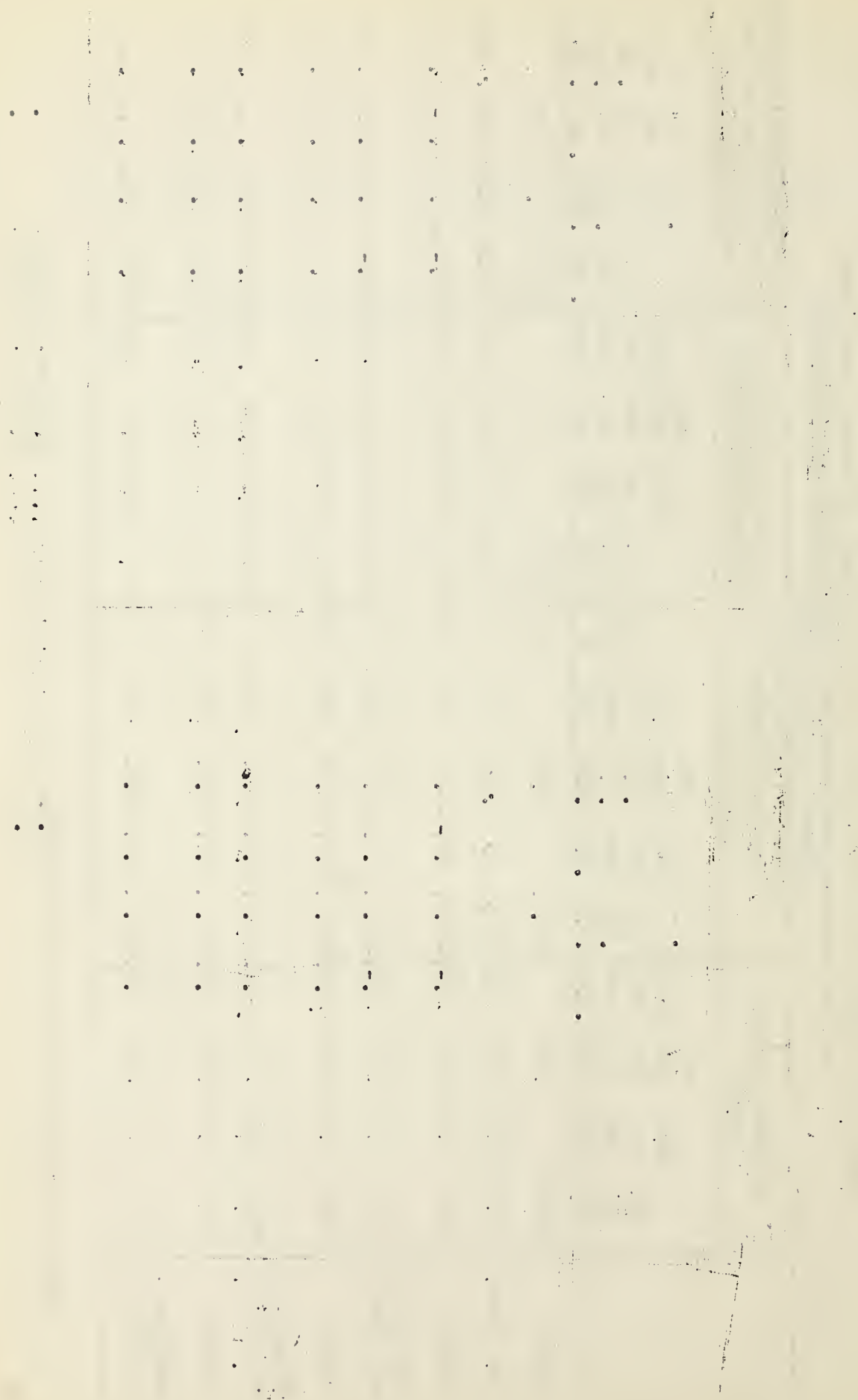


Table 8. Effect of fertilizers on development of wheat seedlings in the presence and absence of Ophiobolus graminis with Edmonton black loam.

Fertilizer Treatment	Soil Treatment	Plant Height			Dry Weight			% Disease		
		Check	Inocu-lation	Differ-ence	Check	Inocu-lation	Differ-ence	Check	Inocu-lation	Differ-ence
Untreated	Sterilized	35.8	24.9	10.9	1.34	0.85	0.49	0.0	52.9	-52.9
	Unsterilized	32.5	16.7	15.8	1.08	0.60	0.48	0.8	81.0	-80.2
	Difference	3.3	8.2		.26	0.25		-0.8	-28.1	
Am. phos. 16-20	Sterilized	36.0	27.5	8.5	1.00	0.97	0.03	0.0	50.5	-50.5
	Unsterilized	35.7	20.8	14.9	1.23	0.74	0.49	1.1	67.7	-66.6
	Difference	0.3	6.7		-0.23	0.23		-1.1	-27.2	
Alfalfa	Sterilized	35.3	24.8	10.5	1.00	0.76	0.24	0.2	52.8	-52.6
	Unsterilized	33.0	26.2	6.8	1.14	0.79	0.35	0.0	52.8	-52.8
	Difference	2.3	-1.4		-0.14	-0.03		0.2	0.0	
Alfalfa, am. phos. 16-20	Sterilized	33.4	26.4	7.0	0.94	0.75	0.19	0.6	48.9	-48.3
	Unsterilized	35.1	25.8		1.10	0.72	0.38	2.1	46.2	-44.1
	Difference	-1.7	0.6		-0.16	0.03		-1.5	2.7	
Straw	Sterilized	38.0	33.4	9.3	1.18	1.14	0.04	0.0	23.1	-23.1
	Unsterilized	33.5	28.2	5.3	1.02	0.79	0.23	0.6	43.6	-43.0
	Difference	4.5	5.2		0.16	0.35		-0.6	-20.5	



Table 8 (continued)

Fertilizer Treatment	Soil Treatment	Plant Height			Dry Weight			% Disease		
		Check	Inocu-lation	Differ-ence	Check	Inocu-lation	Differ-ence	Check	Inocu-lation	Differ-ence
Straw 16-20	Sterilized	36.6	34.1	2.5	1.38	1.14	0.24	0.2	23.7	-23.5
	Unsterilized	36.8	29.3	7.5	1.24	0.79	0.45	0.4	37.7	-37.3
	Difference	-0.2	4.8		0.14	0.35		-0.2	014.0	
Sawdust	Sterilized	36.9	34.2	2.7	1.13	1.06	0.07	0.2	19.3	-19.1
	Unsterilized	32.9	22.4	10.5	0.93	0.69	0.24	1.0	55.6	-54.6
	Difference	4.0	1.8		0.20	0.37		-0.8	-36.3	
Sawdust 16-20	Sterilized	37.7	33.0	4.4	1.26	1.01	0.25	0.0	21.1	-21.1
	Unsterilized	34.3	28.6	5.7	1.10	0.88	0.22	0.2	36.9	-36.7
	Difference	3.4	4.4		0.16	0.23		-0.2	-15.8	

Minimum significant difference between any individuals-- 9.33 cm. height.  
 -- 0.39 gm. weight.



Table 9. Effect of fertilizers on reaction of wheat seedling to Ophiobolus graminis in pot cultures of unsterilized and sterilized recontaminated Fallis gray wooded soil.

	In unsterilized soil				In unsterilized soil infested with Ophiobolus				Unsterilized	In recontaminated sterilized soil				In recontaminated sterilized soil infested with Ophiobolus				Sterilized
	Ht. ave. cm.	Gain or loss cm.	Wt. per pot ave. gms.	Gain or loss gms.	Ht. ave. cm.	Gain or loss cm.	Wt. per pot ave. gms.	Gain or loss gms.	Dis. %	Ht. ave. cm.	Gain or loss cm.	Wt. per pot ave. gms.	Gain or loss gms.	Ht. ave. cm.	Gain or loss cm.	Wt. per pot ave. gms.	Gain or loss gms.	Dis. %
Check	25.0	-	1.08	-	7.0	-	0.24	-	97.1	28.7	-	1.3	-	6.7	-	0.18	-	95.
Am. phos.	28.2	3.2	0.95	-.13	6.5	-0.5	0.24	-	96.7	28.7	-	1.29	-.01	12.2	5.5	0.46	.28	78.
Alfalfa	33.4	8.4	1.55	.47	13.8	6.8	0.52	.28	74.5	32.0	3.3	1.50	.20	16.0	9.3	0.62	.44	74.
Alfalfa am. phos.	34.8	9.8	1.67	.59	11.6	4.6	0.36	.12	80.1	31.1	2.4	1.58	.28	14.6	7.9	0.58	.40	84.
Straw	30.4	5.4	1.18	.10	16.0	9.0	0.65	.41	72.9	39.4	10.7	1.27	-.03	27.5	20.8	1.14	.96	17.
Straw am. phos.	33.6	8.6	1.64	.56	20.6	13.6	1.13	.89	55.5	31.8	3.1	1.85	.55	26.0	19.3	1.36	1.18	37.
Sawdust	25.8	0.8	0.90	-.18	7.9	0.9	0.26	.02	94.1	27.3	-1.4	1.02	-.28	20.9	14.2	0.81	.63	58.
Sawdust am. phos.	29.4	4.4	1.33	.25	8.6	1.6	0.34	.10	93.6	28.8	0.1	1.27	-.03	13.0	6.3	0.55	.37	83.

Minimum significant difference between any individual - 11.61 cm. height  
0.1456 gm. weight.



Table 10. Effect of fertilizers on reaction of wheat seedlings to *Ophiobolus graminis* in pot cultures of unsterilized and sterilized recontaminated Fallis gray wooded soil.

Fertilizer Treatment	Soil Treatment	Av. Plant Height, cm.			Av. Dry Weight per pot, gm.			% Disease		
		Check	In-fested	Difference	Check	In-fested	Difference	Check	In-fested	Difference
Untreated	Sterilized	28.7	6.7	22.0	1.30	0.18	1.12	0.0	95.4	+95.4
	Unsterilized	25.0	7.0	18.0	1.08	0.24	0.84	0.8	97.9	+97.1
	Difference	+3.7	+0.3		+0.22	+0.06		+0.8	+2.5	
am. phos.	Sterilized	28.7	12.2	16.5	1.29	0.46	0.83	0.4	78.0	+77.6
	Unsterilized	28.2	6.5	21.7	0.95	0.24	0.71	0.9	97.6	+96.7
	Difference	+0.5	+5.7		+0.34	+0.22		+0.5	+19.6	
Alfalfa	Sterilized	32.0	16.0	16.0	1.50	0.62	0.88	0.0	74.2	+74.2
	Unsterilized	33.4	13.8	19.6	1.55	0.52	1.03	0.0	74.5	+74.5
	Difference	+0.6	+2.2		+0.05	+0.10		0.0	+0.3	
Alfalfa + am.phos.	Sterilized	31.1	14.6	16.5	1.58	0.58	1.00	0.0	83.6	+83.6
	Unsterilized	34.8	11.6	23.2	1.67	0.36	1.31	1.1	81.2	-80.1
	Difference	+3.7	+3.0		+0.09	+0.22		+1.1	+2.4	
Straw	Sterilized	39.4	27.5	11.9	1.27	1.14	0.13	0.4	17.3	+16.9
	Unsterilized	30.4	16.0	14.4	1.18	0.65	0.53	0.0	72.9	+72.9
	Difference	+9.0	+11.5		+0.09	+0.49		+0.4	+55.6	



Table 10 (continued)

Fertilizer Treatment	Soil Treatment	Av. Plant Height, cm.			Av. Dry Weight per pot, gm.			% Disease		
		Check	In-fested	Difference	Check	In-fested	Difference	Check	In-fested	Difference
Straw + am. phos.	Sterilized	31.8	26.0	5.8	1.95	1.36	0.49	0.0	37.4	+37.4
	Unsterilized	33.6	20.6	13.0	1.64	1.13	0.51	0.0	55.5	+55.5
	Difference	+1.8	+5.4		+0.21	+0.23		0.0	+18.1	
Sawdust	Sterilized	27.3	20.9	6.4	1.02	0.81	0.21	0.0	57.9	+57.9
	Unsterilized	25.8	7.9	17.9	0.90	0.26	0.64	0.9	95.0	+94.1
	Difference	+1.5	+13.0		+0.12	+0.55		+0.9	+37.1	
Sawdust+am. phos.	Sterilized	28.8	13.0	15.8	1.37	0.55	0.72	0.8	83.9	+83.1
	Unsterilized	29.4	8.6	20.8	1.33	0.34	0.99	1.6	95.2	+93.6
	Difference	+0.6	+4.4		+0.06	+0.21		+0.8	+11.3	

Minimum significant difference between any individuals-- 11.61 cm. height  
-- 0.1456 gm. weight



Table 11. Gray wooded soil 2nd pot culture experiment.  
Effect of fertilizers on the reaction of wheat to *Ophiobolus graminis*

	In unsterilized soil				In unsterilized soil infested with <i>Ophiobolus</i>				In recontaminated sterilized soil				In recontaminated sterilized soil infested with <i>Ophiobolus</i>					
	Ht. ave. cm.	Gain or loss cm.	Wt. per pot ave. gms.	Gain or loss gms.	Ht. ave. cm.	Gain or loss cm.	Wt. per pot ave. gms.	Gain or loss gms.	Ht. ave. cm.	Gain or loss cm.	Wt. per pot ave. gms.	Gain or loss gms.	Ht. ave. cm.	Gain or loss cm.	Wt. per pot ave. gms.	Gain or loss gms.	% Dis. Diff.	
Check	27.2	-	.53	-	24.5	-	.46	-	42.8	30.4	-	.60	-	29.5	-	.52	-	20.3
16-20	29.0	1.8	.50	-.03	24.1	-0.4	.44	-.02	42.2	29.6	-0.8	.61	.01	24.0	-5.5	.42	-.10	46.5
Straw rotted 16-20	26.4	-0.8	.48	-.05	23.9	-0.6	.41	-.05	43.8	25.4	-5.0	.54	-.06	25.5	-4.0	.52	.00	27.3
Rotted straw	26.0	-1.2	.45	-.08	24.2	-0.3	.45	-.01	31.2	25.8	-4.6	.44	-.16	26.1	-3.4	.46	-.06	18.3
Unrotted straw 16-20	27.2	0.0	.47	-.06	17.7	-6.8	.31	-.15	78.3	24.1	-6.3	.55	-.05	18.0	-11.5	.35	-.17	71.7
Unrotted straw	25.4	-1.8	.45	-.08	21.7	-2.8	.40	-.06	54.4	26.2	-4.2	.50	-.10	26.2	-3.3	.50	-.02	23.1

Minimum significant difference - 7.79 cm. height  
0.1626 gms. weight.



Table 12. Gray wooded soil 2nd pot culture experiment.  
Effect of fertilizers on the reaction of wheat to *Ophiobolus graminis*

Fertilizer Treatment	Soil Treatment	Plant Height			Dry Weight			% Disease		
		Check	Inocu-lation	Differ-ence	Check	Inocu-lation	Differ-ence	Check	Inocu-lation	Differ-ence
Untreated	Sterilized	30.4	29.5	0.9	0.60	0.52	0.08	0.2	20.5	-20.3
	Unsterilized	27.2	24.5	2.7	0.53	0.46	0.07	1.0	43.8	-42.8
	Difference	3.2	5.0		0.07	0.06		-0.8	-23.3	
16-20	Sterilized	29.6	24.0	5.6	0.61	0.42	0.19	0.3	46.8	-46.5
	Unsterilized	29.0	24.1	4.9	0.50	0.44	0.06	0.0	42.2	-42.2
	Difference	0.6	-0.1		0.11	-0.02		0.3	2.6	
16-20 rotted straw	Sterilized	25.4	25.5	-0.1	0.54	0.52	0.02	0.9	28.2	-27.3
	Unsterilized	26.4	23.9	2.5	0.48	0.41	0.07	0.7	44.5	-43.8
	Difference	-1.0	1.6		0.06	0.11		0.2	-16.3	
Rotted straw	Sterilized	25.8	26.1	-0.3	0.44	0.46	-0.02	1.2	19.5	-18.3
	Unsterilized	26.0	24.2	1.8	0.45	0.45	0.00	1.5	32.7	-31.2
	Difference	-0.2	1.9		-0.01	0.01		-0.3	-13.2	
16-20 unrotted straw	Sterilized	24.1	18.0	6.1	0.55	0.35	0.20	0.7	72.4	-71.7
	Unsterilized	27.2	17.7	9.5	0.47	0.31	0.16	1.1	79.4	-78.3
	Difference	-3.1	0.3		0.08	0.04		-0.4	-7.0	



(Table 12 continued)

Fertilizer Treatment	Soil Treatment	Plant Height			Dry Weight			% Disease		
		Check	Inocu- lation	Differ -ence	Check	Inocu- lation	Differ -ence	Check	Inocu- lation	Differ -ence
Unrotted straw	Sterilized	26.2	26.2	0.0	0.50	0.50	0.00	2.8	25.9	-23.1
	Unsterilized	25.4	21.7	3.7	0.45	0.40	0.05	0.2	54.6	-54.4
	Difference	0.8	4.5		0.05	0.10		2.6	-28.7	
Minimum significant difference				7.79 cm.			0.1626 gm.			



In Fallis Gray Wooded Silt Loam, Second Pot Culture Experiment.

---

Comparison of rotted and unrotted straw applied to Fallis gray wooded soil separately and in combination with ammonium phosphate are shown in Tables No. 11 and No. 12.

Infesting the soil with Ophiobolus graminis produced very large increases in percentage of disease, and this disease was not decreased by any fertilizer but was rather increased by some. The average heights and dry weights were in no cases increased by the fertilizers but were significantly decreased by some.

Significant decreases in dry weights were obtained in infested sterilized recontaminated soil fertilized with unrotted straw plus ammonium phosphate as compared to unfertilized.



EFFECTS OF WHEAT STRAW AND AMMONIUM PHOSPHATE FERTIL-  
IZERS ON REACTION OF WHEAT TO OPHIOBOLUS GRAM-  
INIS IN FALLIS GRAY WOODED SOIL FIELD PLOTS

---

Table 13 shows the effects of wheat straw and ammonium phosphate fertilizers on reaction of wheat to Ophiobolus graminis in field plots of Fallis gray wooded silt loam. The percentage of disease was increased considerably and the yields, both the total weight (grain plus straw) and grain weight, were depressed in each case by artificial infestation. The yields were considerably increased by ammonium phosphate both in the infested and uninfested plots. Ammonium phosphate plus straw gave a slight increase over the check in the uninfested plots, and with the same treatment the infested plot gave a yield comparable to the infested ammonium phosphate plot. Straw alone decreased the yield below the check both in the infested and uninfested plots.



Table 13. Effects of wheat straw and ammonium phosphate on reaction of wheat to Ophiobolus graminis in Fallis gray wooded soil field plots, 1939.

Treatment	Percentage Disease	Av. Yields	
		Grain + straw Air-dry grams per row	Grain Air-dry grams per row
Unfertilized	19	341	16
" Infested	41	100	4
Am. phos.	16	457	21
" Infested	24	300	13
Straw	23	219	10
" Infested	44	87	3
Am. phos. + straw	19	366	17
" + " Infested	28	273	7



THE EFFECTS OF FERTILIZERS ON NITRATES IN  
STERILIZED RECONTAMINATED SOIL AND  
UNSTERILIZED SOIL

---

Edmonton Black Loam

The effects of organic and inorganic fertilizers on nitrate in unplanted cultures are shown in Table no. 14. Alfalfa plus ammonium phosphate gave a larger increase in nitrate than alfalfa or ammonium phosphate in both the sterilized recontaminated and unsterilized Edmonton black loam. Alfalfa seemed slightly superior for the production of nitrates as compared to the ammonium phosphate in unsterilized soil. The reverse was true when alfalfa and ammonium phosphate were compared in steam sterilized recontaminated soil. Nitrate production was low for the duration of the experiment when sawdust or straw were used. Combinations of ammonium phosphate plus straw were used. Combinations of ammonium phosphate plus straw and ammonium phosphate plus sawdust gave increases of nitrates over the organic fertilizers alone, the nitrates produced being similar to the check or approximately one-half that produced when ammonium phosphate was used alone. The production of nitrate was lowest when sawdust was added alone.

There appeared to be no effect on nitrate accumulation when Ophiobolus graminis was added.

The unsterilized soil was much superior to the steam sterilized recontaminated soil for the production of nitrates.



Fallis Gray Wooded Silt Loam

In general the results are somewhat the same in Fallis gray wooded silt loam, Table No. 15, as in the Edmonton black loam. Alfalfa gave a larger increase in nitrate than ammonium phosphate, and alfalfa plus ammonium phosphate gave the largest increase, both in the unsterilized and the sterilized contaminated. The steam sterilized and recontaminated soil was much lower in nitrate than the unsterilized. Straw and sawdust decreased the production of nitrates to traces in unsterilized soil. Straw and sawdust in combination with ammonium phosphate gave double the nitrate content of the check, the straw plus ammonium phosphate being somewhat superior to the sawdust plus ammonium phosphate.

The addition of sawdust and straw alone and in combination with ammonium phosphate decreased nitrate production to a trace in sterilized recontaminated soil.



Table 14. Effect of fertilizers on nitrification in unsterilized and recontaminated sterilized Edmonton black loam soil, in the presence and absence of Ophiobolus graminis.

	Unsterilized soil						Unsterilized soil in- fested with <u>Ophiobolus</u>						Recontaminated sterilized soil						Recontaminated ster- ilized soil infested with <u>Ophiobolus</u>					
	Weeks incubated			Weeks incubated			Weeks incubated			Weeks incubated			Weeks incubated			Weeks incubated			Weeks incubated			Weeks incubated		
	At start	2	4	6	At start	2#	3	4	6	At start	2	4	6	At start	2	4	6	At start	2#	3	4	6		
Check	2.6	54	104	109	3	54	81	93	122	trace	7	30	trace	6	13	40								
Am. phos.		102	218	200		102	132	98	186		4	21	77		4	14	25	104						
Alfalfa		100	169	250		100	167	227	186		trace	8	18		trace	9	19	79						
Alfalfa am. phos.		185	139	365		185	208	270	323		5	38	204		5	23	49	91						
Straw		11	39	39		11	32	28	44		trace	9	13		trace	5	5	12						
Straw am. phos.		61	125	156		61	107	96	152		6	22	54		6	15	15	44						
Sawdust		13	23	19		13	16	9	15		trace	14	11		trace	trace	trace	20						
Sawdust am. phos.		32	122	99		32	66	102	78		3	4	44		3	9	12	46						

#Infested with Ophiobolus graminis after 2 weeks' incubation.



Table 15. Effect of fertilizers on nitrification in unsterilized and recontaminated sterilized Fallis gray wooded soil, in the presence and absence of Ophiobolus graminis.

Nitrate-nitrogen (parts per million)																			
Unsterilized soil					Unsterilized soil infested with <u>Ophiobolus</u>					Recontaminated sterilized soil					Recontaminated sterilized soil infested with <u>Ophiobolus</u>				
Weeks incubated At start					Weeks incubated At start					Weeks incubated At start					Weeks incubated At start				
2	4	6	2	4	6	2	4	6	2	4	6	2	4	6	2	4	6		
Check	11	22	33	24	11	22	28	26	25	7	14	17	23	7	14	9	10	17	
Am. phos.		23	69	114		23	50	65	93		11	18	33		11	7	12	17	
Alfalfa		96	152	216		96	135	114	139		10	33	56		10	128	23	94	
Alfalfa + am. phos.		119	179	295		119	239	179	218		11	45	91		11	trace	21	150	
Straw		trace	trace	16		trace	trace	trace	trace		trace	trace	trace		trace	trace	trace	trace	
Straw + am. phos.		12	38	53		12	32	42	71		trace	trace	15		trace	trace	trace	8	
Sawdust		trace	trace	4		trace	trace	trace	trace		trace	trace	trace		trace	trace	trace	trace	
Sawdust + am. phos.		5	22	29		5	16	29	42		4	trace	8		4	trace	trace	trace	

#Infested with Ophiobolus graminis after 2 weeks' incubation.



## DISCUSSION.

Bacterial counts in sterilized recontaminated soils of Edmonton and Fallis were much larger after incubation than in the unsterilized, while in Gros Ventre soil there was no consistent difference. The Gros Ventre is intermediate in fertility compared to Edmonton and Fallis soils. Malowany (14) found that ammonification, nitrification, water soluble sulfate, and the easily soluble and water soluble phosphates were increased after incubation in steam sterilized recontaminated soils, and that the greater differences were to be found in the soils containing the greater amounts of organic matter. From these results one would expect intermediate counts for Gros Ventre soil.

The ~~fungi~~ counts <sup>of fungi</sup> were decidedly increased after incubation in the three sterilized recontaminated soils, the greatest increase in numbers being obtained in the Gros Ventre soils. The decided increase in steam sterilized recontaminated soils in numbers of fungi found in Gros Ventre soil may be one explanation of why the bacteria counts were not increased consistently by this treatment. The more available nutrients and the organic matter may have been used by the fungi at the expense of the bacteria. Malowany (14) also found that there is very little difference in pH of these three soils, and that steam sterilization apparently does not affect the hydrogen ion concentration; therefore the pH of the soil is not likely the factor responsible for the great development of fungi in sterilized recontaminated soil. Russell and Hutchinson (19) found that partial sterilization of a soil by heat or chemicals resulted in decreased



numbers of micro-organisms for a few days; then there was an increase to greater numbers than in untreated soil.

Since dilutions were not carried high enough to determine the approximate protozoan count of these soils, we did not obtain a true picture and no conclusions can be drawn from the data obtained. Protozoa were present in the three soils investigated, and the more fertile Edmonton black loam probably contains more than 100,000 per gram of soil. The other two soils, Fallis gray wooded and Gros Ventre, had fewer: probably between 10,000 and 100,000. Active organisms were not obtained in all plates; the majority were cysts or dead organisms.

Pure cultures of fungi vary in ammonifying power, which is well illustrated ~~from~~<sup>by</sup> the data presented. Coleman (2) found that the activity of fungi and their relative positions was changed with different temperatures. The data presented were obtained from cultures that were subjected to room temperatures, consequently there were fluctuations in temperature. Therefore, the data obtained from the investigations conducted might be quite different if the experiments had been conducted at different temperatures. Nevertheless, with fluctuating temperatures we have conditions that are similar to those found in nature, which would justify the work being carried out under these conditions, and, when the relative positions of the cultures were maintained, differences in ammonifying power throughout a considerable range of temperature were indicated. Coleman (2) found that the quantity of organic matter and the type of soil had a marked effect on the activity of fungi. From the data obtained with pure cultures of fungi in Edmonton high organic matter soil, and the very infertile low organic matter Fallis



soil, his findings have been substantiated. There was very little ammonia produced by the cultures in Fallis soils and the differences were not great enough between cultures to state that there was any marked difference in ammonifying power of the cultures, which was not the case in Edmonton black loam. We may therefore conclude that pure cultures of fungi vary in their ammonifying power, under the conditions of the experiments, and that soil type affects their activity to a marked extent.

Semeniuk (21) found that there was no definite relationship between organic matter content, nitrogen content, and the carbon dioxide evolved from three Alberta soils. The indications from the experiments on the carbon dioxide evolution of sterilized recontaminated soils compared to the unsterilized are contradictory to those of Semeniuk, but agree with those of Wollny (33). Wollny found a correlation between organic matter of soil and carbon dioxide production. His results are not exactly comparable as he added organic matter to the soil in varying amounts. The form of organic matter added was horse manure which is a fresh form and not comparable with that found in the soil. A comparison of the data of unsterilized soils indicates that the Edmonton high organic matter soil has a greater production of carbon dioxide than the infertile low organic matter Fallis soil, while the Gros Ventre soil, intermediate in organic matter, is also intermediate in carbon dioxide production. Stoklassa et al (24, 25) found that soil fertility was an important factor in the carbon dioxide evolved, which is in direct agreement with the data presented in comparisons of unsterilized soils in this investigation. They also found that sterilized soil produced no carbon dioxide, and



that when conditions were brought about that favored biological activity there was greater evolution of carbon dioxide than from unsterilized soil. It has been found in this investigation that steam sterilization and recontamination of soils has resulted in decided increases in the microflora. We may therefore conclude that increased carbon dioxide production in steam sterilized recontaminated soils as compared to unsterilized is a result of greater biological activity.

In work done with pure cultures of fungi in Edmonton black loam, it has been found that fungi vary in their carbon dioxide producing ability. With the exception of the non-antagonistic fungus No. 44, Penicillium sp., there was a correlation between carbon dioxide and ammonia production by pure cultures of fungi. Fungus No. 44, Penicillium sp., was a low producer of carbon dioxide and a medium producer of ammonia, suggesting that this organism attacks the protein fraction of the soil organic matter more than some of the other fungi tested. Gainey (6) also found that there was a correlation between carbon dioxide and ammonia production in soils. As in the ammonification experiments, none of the non-antagonistic fungi tested were high carbon dioxide producers.

Schreiner et al (23) found that steam sterilization partly decomposes organic matter which is then more easily attacked than undecomposed organic matter. Malowany (14) has substantiated this finding as he found greater production of ammonia occurred in steam sterilized soils recontaminated with the normal flora than in the corresponding unsterilized soils. From the foregoing findings one would expect to obtain a greater quantity of displaceable ammonia as a result of steam sterilization, par-



ticularly in the soils high in organic matter. The results presented verify this assumption, for the Edmonton soil high in organic matter had originally most ammonia and showed the greatest increase from steam sterilization. The Fallis gray wooded soil deficient in organic matter contained very little ammonia and showed the least increase from the treatment. The Gros Ventre brown prairie soil was intermediate in organic matter and showed an intermediate increase immediately following steam sterilization.

Alfalfa, straw and sawdust were used alone and in combination with ammonium phosphate in unsterilized and recontaminated sterilized Edmonton soil, half of the series being infested with Ophiobolus graminis.

In general the fertilizers decreased disease severity. There was less disease in the sterilized recontaminated pots than in the unsterilized, particularly where straw and sawdust had been applied alone and in combination with ammonium phosphate. Increases in weight produced by fertilizers were not significant, increases and decreases being obtained from their use. In uninfested soils there was no significant increases or decreases for wheat plant height. Infested soils gave significant increases in plant height for straw alone and in combination with ammonium phosphate, also for alfalfa alone and with ammonium phosphate. The results indicate that the fertilizers used are not beneficial in the already fertile Edmonton soil when the plants are not in contact with the disease producing organism. From a study of the nitrification data there are no indications that high nitrates are beneficial, as alfalfa gave a high



accumulation of nitrates while straw was comparatively low. A possible explanation for the sterilized recontaminated soil having less disease may be obtained from the microflora studies. There would be a greater microflora population which would probably be composed of greater numbers of antagonistic organisms (12).

#### Fallis Gray Wooded Silt Loam

Disease severity was decreased to some extent by most of the fertilizers used in pot culture experiments, the greater reduction taking place in recontaminated sterilized soils. The fertilizer expressing the greatest degree of control was straw alone and in combination with ammonium phosphate. Plant heights were in general increased, significant increases being obtained only with straw and straw plus ammonium phosphate in some of the treatments. All fertilizers gave significant weight increases in recontaminated infested soils, and generally with straw alone and alfalfa alone and in combination with ammonium phosphate for the other soil treatments.

A similar pot culture experiment to the above was conducted comparing rotted and unrotted straw. The rotted straw was comparable to the straw in the previous experiment; that is, it was added three weeks before seeding, and the unrotted straw was added immediately before seeding. The rotted straw gave similar control of disease severity as in the first experiment. The unrotted straw did not, but rather increased the disease. These results would indicate that the stage of decomposition of the amendments affected the results and this was indicated also in field plots at Fallis. Straw was added one week before seeding,



with the result that infection was not suppressed and yield was decidedly decreased. Ammonium phosphate in these field trials inhibited disease to some extent and increased yields. Ammonium phosphate plus straw was intermediate in suppressing disease and increasing yields.



### SUMMARY OF RESULTS.

Under the conditions of the experiment reported in this investigation, steam sterilization and recontamination has an effect on the microbiological population, the biochemical activity and the physical properties of the three Alberta soils under investigation, when comparisons are made with the unsterilized.

Plate counts of the numbers of bacteria in the Edmonton black loam and Fallis gray wooded silt loam were much larger after two weeks incubation in the steam sterilized recontaminated soil than in the original soil. Gros Ventre brown prairie loam did not give significant increases from the treatment.

Plate counts of the numbers of fungi were much larger in all cases in the sterilized recontaminated soil than in the unsterilized, Gros Ventre brown prairie loam having by far the greatest increase in numbers.

Protozoa were present in all soils; there appears to be no significant difference between steam sterilized, recontaminated and unsterilized soils.

Ammonia production of pure cultures varied in Edmonton black loam. There appears to be no close correlation between antagonism and non-antagonism to Ophiobolus graminis and ammonia production. With the exception of Fungus No. 44, Penicillium sp., none of the non-antagonistic fungi tested were strong ammonifiers.

In the relatively infertile Fallis gray wooded silt loam, the fungi tested showed no pronounced differences in ammonifying power.

The fungi tested varied as to their carbon dioxide production in sterilized Edmonton black loam. The most antagonistic



organism was a high producer of carbon dioxide, while the non-antagonistic were low to intermediate producers of carbon dioxide.

With the exception of the non-antagonistic fungus No. 44, Penicillium sp., there was some correlation between carbon dioxide and ammonia production in Edmonton black loam. Fungus No. 44, Penicillium sp., was a low producer of carbon dioxide and a comparatively high ammonifier.

There was greater carbon dioxide production in the sterilized Edmonton black loam and Gros Ventre brown loam recontaminated with original soil than in the corresponding unsterilized soils. However, in the corresponding case of the relatively infertile Fallis gray wooded silt loam, significant differences were not obtained.

An immediate increase was obtained in displaceable ammonia when the three soils were sterilized by steam under pressure.

Apparently the fertilizers, alfalfa, straw, and sawdust, alone and in combination with ammonium phosphate, were beneficial in increasing crop yield and decreasing disease severity in pot culture experiments when Edmonton black loam was infested with Ophiobolus, straw and straw plus ammonium phosphate being superior to others used. All fertilizers gave an increase in wheat yields but these were not generally significant. The fertility of recontaminated sterilized soils had been increased by the treatment with a result that additions of fertilizer were of little value for increasing yields.

Infesting Fallis gray wooded silt loam with Ophiobolus graminis produced large increases in the percentage of disease in pot culture experiments. Alfalfa, straw, and sawdust, alone and in combination with ammonium phosphate apparently decreased the disease to some extent, but straw alone and straw plus ammonium



phosphate were superior to the others used. All fertilizers except ammonium phosphate gave increases in yield, the least response being obtained in recontaminated sterilized soil. Apparently the fertilizers are less beneficial where the fertility has probably been increased by sterilization and recontamination.

Unrotted straw alone and in combination with ammonium phosphate apparently did not decrease the disease in Fallis gray wooded silt loam. Rotted straw in sterilized recontaminated soil decreased disease severity. Infesting the soil with Ophiobolus graminis had no effect on plant height in the case of sterilized recontaminated soil.

In field plots at Fallis the yields were increased by ammonium phosphate in both the infested and uninfested plots. Straw depressed the yields and ammonium phosphate plus straw gave smaller increases than ammonium phosphate alone, especially in the uninfested plots.

No definite conclusions can be stated as to the effects of fertilizers on the severity of the "take-all" disease of wheat. However, indications are that partially rotted straw alone and in combination with ammonium phosphate will give some measure of control. Crop responses to fertilizers are apparently correlated with original fertility of the soil.

Nitrates were generally higher in unsterilized soil than in recontaminated sterilized for all fertilizers used. Infesting the soil with Ophiobolus had no apparent effect on nitrates. Alfalfa gave a larger increase of nitrate than ammonium phosphate, and alfalfa plus ammonium phosphate gave the largest increase. Straw and sawdust generally depressed nitrates but when used in



combination with ammonium phosphate nitrates were increased.

There was in general no close correlation between crop yields and the available nitrates but the sawdust decreased the yields as well as the nitrates in certain cases in the gray soil.



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# REFERENCES

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1. BROADFOOT, W.C.  
Does the wheat plant become more susceptible to the foot rotting fungi with increasing age? Amm. Rpt. Dominion Botanist, Dept. of Agric., Canada. 1930.
2. COLEMAN, D.A.  
Environmental factors influencing the activity of soil fungi. Soil Sci. 2: 1-65. 1916.
3. DIXON, ANNIE  
Soil protozoa; the growth on various media. Ann. Appl. Biol. 24: 442-456. 1937.
4. FELLOWS, H.  
Studies of certain soil phases of the wheat take-all problem. Abstract. Phytopath. 19--. 103. 1929.
5. FELLOWS, H. -  
The influence of oxygen and carbon dioxide on the growth of Ophiobolus graminis in pure culture. Jour. Agr. Res. 37: 349-355. 1928.
6. GAINNEY, P.L. and NELLER, J.R.  
Parallel formation of carbon dioxide, ammonia and nitates in soil. Soil Sc. 7: 293-311. 1919.
7. GARRETT, S.D.  
Soil conditions and the take-all disease of wheat. Ann. Appl. Biol. 23: 667-699. 1936.
8. GARRARD, E.H. and LOCHHEAD, A.G.  
Relationships between soil micro-organisms and soil-borne plant pathogens. A Review. Sci. Agr. 18: 719-737. 1938.
9. HARPER, H.J.  
Method of determination of nitrates. Indust. and Engng. Chem. 16: 180-183. 1924.
10. HENRY, A.W.  
The natural microflora of the soil in relation to the foot-rot problem of wheat. Can. Jour. Res. 4: 69-77. 1931.
11. HENRY, A.W.  
Influence of soil temperature and soil sterilization on the reaction of wheat seedlings to Ophiobolus graminis Sacc. Can. Jour. Res. 71: 198-203. 1932.



12. LUDWIG, R.A.  
Studies of the microbiology of sterilized soil in relation to its infestation with plant pathogens.  
Unpublished thesis, University of Alberta. 1939.
13. MACHACEK, J.E.  
Preliminary investigations on the effect of excessive soil salinity on the incidence of cereal root-rot.  
Sci. Ag. 17: 215-224. 1936.
14. MALOWANY, S.N.  
Some effects of steam sterilization on physical, chemical and biological relationships of soils.  
Unpublished thesis, University of Alberta. 1938.
15. McLEAN, H.C. and WILSON, G.W.  
Ammonification studies with soil fungi. N.J.Agr. Exp. Sta. Bul. 270. 1914.
16. MacLEAN, W. and ROBINSON, G.W.  
A new method for the determination of ammoniacal nitrogen in soils. J. Agr. Sc. 14: 548-554. 1924.
17. NEAL, D.C., WEBSTER, R.E. and GUNN, K.C.  
Growth of the cotton root-rot fungus in synthetic media and the toxic effects of ammonia on the fungus.  
J. Agr. Research, 47: 107-117. 1933.
18. PORTER, C.L. and CARTER, J.C.  
Competition among fungi. Bot. Rev., 4: 165-182. 1938.
19. RUSSELL, E.J. and HUTCHINSON, H.B.  
The effects of partial sterilization on the production of plant foods. J. Agr. Sc. 3: 111-144. 1908-10.
20. SANFORD, G.B. and BROADFOOT, W.C.  
Studies on the effects of other soil-inhabiting micro-organisms on the virulence of Ophiobolus graminis Sacc.  
Sci. Agr. 11: 512-528. 1931.
21. SEMENUK, G.  
Studies of the influence of soil composition on the growth and nutrition of certain fungi causing foot- and root-rot of wheat. Unpublished thesis, University of Alberta. 1934.
22. SCOTT, W.W.  
Standard method of chemical analysis. Fourth ed., Vol. 2.  
D. Van Nostrand Co., New York. 1925.
23. SCHREINER, O. and LATHRUP, E.C.  
The chemistry of steam heated soil. U.S.D.A. Agr. Bur. Soils Bul. 89. 1912.



24. STOKLASA, J. and ERNST, A.  
Ueber den Ursprung die Menge und die Bedeutung des  
Kohlendioxyds im Boden. Central. Bakt. 11. Abt. 14. 1905.
25. STOKLASA, J. and ERNST, A.  
Ueber den Ursprung die Menge und die Bedeutung des Kohlen  
dioxyds im Boden. Ztsehr. Zuckrindue Bohmen, 31: 291-401.  
1911.
26. VANTERPOOL, T.C.  
Studies of browning root-rot of cereals. Can. Jour. Res.  
Sec. C. 3:220-250. 1935.
27. VAN SUCHTELEN, F.H.H.  
Ueber die Messung der ebenstatigflkeit der aerobiotischen  
Bakterien im Boden durch die Kohlens aure produktion.  
Central. Bakt. Abt. 2: 28. 45-63. 1910.
28. WAKSMAN, S.A.  
Associative and antagonistic effects of micro-organisms.  
I. Historical review of antagonistic relationships.  
Soil Sc. 43: 51-68. 1937.
29. WAKSMAN, S.A.  
Soil fungi and their activities. Soil Sc. 2: 103-156.  
1916.
30. WAKSMAN, S.A. and FRED, E.B.  
Laboratory manual of general microbiology. McGraw-Hill  
Book Co. New York. 1928.
31. WAKSMAN, S.A.  
Principles of soil microbiology. Chapters 21 and 29.  
Williams and Wilkins Co. Baltimore. 1927.
32. WAKSMAN, S.A. and STARKEY, R.L.  
Partial sterilization of soil, microbiological activities  
and soil fertility. Soil Sc. 16: 137-156, 247-268. 1923.
33. WOLLNY, E.  
"Die zersetzung der organischen stoffe."









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